Maternal Adversity, Vulnerability and Neurodevelopment (MAVAN) Project

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Research Project

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I. Introduction.

Objectives: The probability of chronic illness is strongly influenced by environmental conditions prevailing during development (Hertzman 1999). The frequency of heart disease, diabetes, and mental illness is substantially greater amongst those born and raised in poverty, regardless of social class in adulthood. Adversity associated with poverty produces incomplete, dysfunctional families often rife with domestic violence, malnutrition, drug use, child abuse and neglect. Reproduction within this context is associated with maternal stress, increased risks for infection and thus pre-term labour, perinatal deaths, birth insults, poor nutrition for mother and offspring, and serious compromises in the quality of parent-child interactions. These conditions define high-risk families. For the child, these conditions bear the potential to influence the development of physiological systems that regulate metabolism and cardiovascular function, as well as neural processes underlying emotion and cognition. These effects form the basis for vulnerability for illness.

Perhaps the most dramatic illustration of the relation between adversity in early life and the risk for later illness emerges from studies on birth weight, early growth and health in adulthood. Individuals that were small for gestational age, suggesting some degree of intrauterine growth restriction, are at significantly greater risk for type II diabetes, visceral obesity and hypertension (collectively referred to as the metabolic syndrome). The major predictors of fetal growth restriction and pre-term labour, are maternal stress, infections, malnutrition, and tobacco/alcohol consumption. These risk factors are far more prevalent in low socio-economic status (SES) families and, predictably, low SES is a major risk factor for both intrauterine growth retardation (IUGR) and pre-term labour.

It is increasingly clear that growth restriction also predicts an increased risk for mood disorders (Thompson et al. 1997, and see Kelly et al. 2001). Additionally, the risk factors (i.e., maternal stress, tobacco/alcohol use, nutritional deprivation) for IUGR are also the major predictors for Attentional Deficit Disorder (ADD) and, predictably, ADD is far more prevalent in low SES environments (Biederman et al., 1990, 1995; Barkley 2000). Follow up studies of growth retarded infants reveal evidence for problems associated with attention and impulse control, functions that are heavily dependent on the hippocampus and prefrontal cortex. These same brain regions are also among the major sites implicated in both depression and anxiety disorders. Interestingly, growth restriction is also associated with increased behavioural inhibition, shyness and timidity in childhood, all of which appear to be predictors of mood disorders in later life. Studies with primate and rodent models show that the development of the hippocampus is impaired by fetal glucocorticoid exposure or by prenatal stress, and glucocorticoids are a mechanism for fetal growth restriction.

Glucocorticoids are a major class of stress hormones and the release of these hormones is activated by stress, as well as by protein deprivation, tobacco and alcohol. Thus, we propose *that impairments in the development of the hippocampus and prefrontal cortex serve as the mechanism for the relation between fetal growth restriction and impaired cognitive and emotional development.*

These studies suggest that endocrine conditions that promote fetal growth restriction can also compromise neural development. However, follow-up studies with even very low-birth weight babies reveal an impressive level of variability in cognitive outcomes. Evidence from clinical intervention studies with high-risk infants and developmental neurobiology suggests that neurocognitive development is influenced by the quality of the postnatal environment. Of particular relevance are the results of studies with primate and rodent models indicating that nurturing forms of maternal care can enhance neuronal survival and synaptic growth in regions such as the hippocampus (see below). Thus we propose *that the developmental outcomes associated with fetal adversity, reflected in growth retardation, are determined by the quality of the postnatal environment.*

Longitudinal studies reveal early life markers of poor health outcomes over the lifespan that include low birth weight, irritable temperament in early childhood and poor frustration tolerance, parental stress, attentional deficits and even increased blood pressure. These conditions can be quantified and we propose to use such measures in the development of longitudinal studies on the interactions between prenatal and postnatal environmental conditions in predicting health outcomes. **Specifically, we will investigate influences of the early environment (prenatal and early neonatal) on neuropsychological development. Our focus for the study of postnatal environment is on maternal well-being and mother-infant interactions. Hence our measures include not only functional assessment of the offspring, but also measures focusing on the mother. We feel that this study will provide a crucial basis for the development of focused, early intervention programs.**

Adverse Perinatal Environment: Development of Disease

There is a dramatic increase in the risk for coronary heart disease, obesity and diabetes (metabolic syndrome) among individuals who were small for gestational age, a condition that implies some measure of intrauterine growth retardation (IUGR) and adverse fetal environment (Matthews 2001; Barker 2002; Seckl et al. 1999). The health risk appears linked to the programming effects on gene expression, especially that of hepatic genes. The conditions of risk associated with impoverished fetal life are not restricted to cardiovascular or metabolic disease. Adverse intrauterine environment has also been linked to neurodevelopmental disorders. Both prematurity and IUGR

are consistently associated with cognitive impairments; the existing data suggest that cognitive outcomes are more seriously compromised by growth restriction than by prematurity (Lagercrantz 1997). Studies using more global measures of cognitive development, such as IQ tests, reported significant impairments among IUGR children (e.g., Breslau et al. 1996; Fattal-Valevski et al. 1999; Leitner et al. 2000;). van der Reijden-Lakeman et al. (1997) found that at 2 years of age, IUGR children were impaired on tests of divided, focused and sustained attention. On such tests, the IUGR population was less accurate and more impulsive than controls. These findings suggest that low-birth weight children might be at greater risk for the development of Attentional Deficit Disorder (ADD). Indeed, the risk factors for IUGR, including maternal stress, maternal psychopathology, protein malnutrition, tobacco/alcohol consumption are identical to those for ADD. Likewise, maternal stress during gestation is a predictor of both fetal growth restriction and negative temperament (irritability, negative emotionality) and anxiety in children (O'Connor et al. 2000; Glover et al. 2002). Behavioural disorders, characterized by poor impulsive control, shyness, timidity and negative temperament (greater frequency of negative emotional states) are positively related to the degree of growth restriction (e.g., Szatmari et al. 1990; Pharoah et al 1994; Sykes et al. 1997).

Adverse fetal environment affects the risk for chronic illness via direct and indirect pathways. In the case of the metabolic syndrome, direct effects include modification of the expression of enzymes involved in fat (PEPCK) and glucose (glucose kinases) metabolism. Intellectual and emotional development is affected by direct early environmental effects on neuronal proliferation and synaptogenesis in regions such as the hippocampus, amygdala and the frontal cortex. Indirect effects on metabolic and neurological function include programmed modification of neuroendocrine function, particularly the hypothalamo-pituitary-adrenal (HPA) axis, throughout the lifespan. Indeed, many of the pathologies associated with the metabolic syndrome are identical to those observed in patients with Cushing's syndrome (hypersecretion of cortisol; see McEwen et al. 1999; Lupien & Meaney 2001 for reviews). Furthermore, Cushing's patients are at increased risk of depression and cognitive impairment; both directly linked to dysregulation of HPA function. These observations indicate the central role of HPA function in perinatally programmed disease. In rodents, fetal adversity permanently increases corticotropin-releasing factor expression in the hypothalamus and amygdala; such effects appear to be mediated by alterations in the hippocampus and prefrontal cortex (Meaney 2001). These changes are linked to life-long enhancement of stress reactivity, which serves to exacerbate the risk of developing metabolic and neurological disorders.

Fetal growth retardation is associated with problems of impulse control and attention. The preliminary results of recent imaging studies of low-birth weight children reveal significantly decreased hippocampal volume. Indeed,

alterations in hippocampal and prefrontal development might explain the unusually high level of comorbidity between attentional deficits - impulse control disorders and affective disorders. The hippocampus and prefrontal cortex are also critical structures for impulse control and emotional states such as fear. Importantly, each of the risk factors for IUGR is known to increase fetal adrenal release of glucocorticoids. Thus adversity during fetal development increases exposure to glucocorticoids, which are known to promote growth restriction and compromise neural development, resulting in vulnerability for both cognitive and emotional disorders (Fig. 1). For example, fetal exposure to glucocorticoids decreases neuronal proliferation in the hippocampus (Ono et al., 1989; McEwen 1999; Liu & Matthews 2001). Interestingly, fetal dexamethasone administration in humans for congenital adrenal hyperplasia was associated with increased shyness and timidity in children. Both anxiety disorders and depression are accompanied by significant decreases in hippocampal volume and, in the case of depression, dramatically reduced volume and activity in the prefrontal cortex. The hippocampus and the prefrontal cortex are primary targets for both antidepressant and anti-anxiety medications.

The influence of environmental adversity on offspring development is not limited to the prenatal period. There is clear evidence for the importance of postnatal poverty and family dysfunction on child development and health. Importantly, the effects of socioeconomic status (SES) on emotional and cognitive development in children are mediated by variations in parental care (Conger et al., 1994; McLoyd et al. 1998). Indeed, when parental care factors are statistically controlled, the effect of SES is eliminated. Likewise, low SES is associated with elevated cortisol levels in children, and these effects are mediated by parental factors. This suggests that stress of poverty, which is linked to increased risk of adverse fetal development, also compromises the quality of childcare. In rodent and nonhuman primate models, mother-pup interactions regulate neurodevelopment and disruptions to mother-infant interactions are associated with impaired neurodevelopment and exaggerated HPA and emotional responses to stress (Meaney 2001).

The influence of the postnatal environment is a sword that can, potentially, cut both ways. In the rat, more intense forms of maternal care, characterized by increased pup licking, promote hippocampal synaptic development and dampen HPA responses to stress (Meaney 2001). In the rat, maternal licking causes an increase in neurotrophic factor expression, enhancing both neuronal survival and synaptic outgrowth (Liu et al. 2000; Bredy et al. in press). Increased maternal licking over the first week of life is also directly linked to more modest HPA and behavioral responses to stress (Francis et al., 1999; Caldji et al., 1998, in press). Likewise, in humans, nurturing family environments dampen stress reactivity and promote resistance to chronic illness. Such findings raise the possibility that conditions of the postnatal environment could offset or reverse the risk created during

fetal development. Indeed, in animal studies there is clear evidence that postnatal enrichment, for example, can serve to eliminate the effects of fetal adversity. This issue is obviously of profound significance for the development of early intervention studies.

II. Hypotheses.

Statement of the Problem: There is increasing evidence that many forms of chronic illness in child- and adulthood have perinatal origins. Developmental studies in both human and nonhuman subjects, have only very rarely examined the effects of environmental events at one stage of development in the context of previous experience.

Hypotheses: There is significant interaction between the prenatal and postnatal environment in programming child- and adulthood disease, and that careful manipulation of the infant environment can ameliorate or reverse the negative health outcomes associated with adverse intrauterine experience. *We hypothesize that the effects of fetal adversity on neurodevelopment are determined by the quality of the postnatal environment, particularly by mother-infant interactions. Moreover, we propose that the quality of mother-infant interactions is strongly influenced by maternal emotional well-being.*

III. Methods

Participants. Our approach will be to develop a human cohort derived from a study already nearing completion in Montreal (The Montreal Prematurity Study funded by the March of Dimes; PI: Dr. Michael Kramer). Dr. Kramer is a collaborator on the MAVAN project, and Drs. Meaney and Lydon are collaborators on the Prematurity Study. The Prematurity study involves measures of psychosocial stress, nutrition, and family and community living conditions as predictors of gestational length, which is the outcome measure in this project. This project will recruit 5000 women at 13-15 weeks gestation, of which approximately 4000 have been recruited and will have given birth before the start of our project, leaving a population of 1200 potential subjects. Currently, 100 women each month are assessed at 24-26 weeks gestation. There is currently in place an infrastructure for follow-up of women from the Montreal Prematurity Study.

The cohort for the current MAVAN project will be derived from the same population used in the Prematurity Study. In essence the MAVAN Project will serve as a follow-up study derived from the Prematurity Study focusing on a substantially smaller population. The strategy for the MAVAN Project is to take advantage of the multivariate assessment of prenatal adversity in The Montreal Prematurity Study. We will follow infants born at normal weight (controls, n= 120; 3000-3500 g at birth) and low-

birth weight babies exhibiting evidence for intrauterine growth retardation (IUGR, n= 120; 2200-2750 g at birth). These groups are based on the recently published Canadian standards for fetal growth (Kramer, M. S., R. W. Platt, S. W. Wen, K. S. Joseph, A. Allen, M Abrahamowicz, B. Blondel, and G. Bréart. 2001. "A new and improved population-based canadian reference for birth weight for gestational age." Pediatrics 108:), which indicates that the birth weight of 2,750 g represents the 10th percentile, with a weight of 3750 g at the 70th percentile. Our intent is to avoid extreme cases of growth retardation (i.e., < 2,200 g). All babies in this study will be born at > 37 weeks gestation to avoid the complication of prematurity and growth restriction. We will recruit two cases for each case (i.e., two infants of normal birth weight for each infant that is small for gestational age).

The method of recruitment for the MAVAN Study will follow the same procedures as another CIHR-funded follow-up study of the Prematurity Study. The New Mothers Study (John Lydon, PI, M Kramer, L Seguin, S Lupien, and M Meaney co-investigators) examines the physical and psychological well being of mothers during the first year post-partum with particular attention to mothers of babies born with health problems and mothers of low socio-economic status. The New Mothers Study is in its fourth year. Letters are sent to the mothers about the follow-up to the Prematurity Study with a rejection card and postage paid envelope. Women who do not wish to receive a phone call for the follow-up study simply put the rejection card in the envelope and in the mail. About 2 women per month choose this option. With a 9% prevalence rate of IUGR, we can expect that 126 IUGR babies from the Montreal Prematurity Study will be available as potential participants. Current recruitment rates (~70% participation) suggest that we will obtain a sample of 88 IUGR children and their mothers. Two, normal weight for age babies born on the same or closest day to the IUGR baby will be used as controls with a target of 252. Based on the differential prevalence rates of IUGR among low and high SES mothers, we can expect a sample of 31 low SES mothers and babies in the IUGR group and 57 high SES. Because SES (and associated chronic stressors and depression) may have an adverse effect on child development mediated by postnatal growth delay rather than by IUGR, we will oversample low SES babies who are normal weight for age at birth and consider their postnatal growth as an additional factor predicting cognitive development.

Overview

The measures described here assess the following domains: general development, attention/ impulsivity, social/emotional development, temperament, and basal and stimulated hypothalamic-pituitary-adrenal activity. Methods include measures of parental reports of infant behaviour;

direct testing of the infant, questionnaires for the mother, and observation of mother and infant. Direct testing of the infant occurs with the mother present up to the age of 36 months. At 18-months, 30-45 minutes of maternal testing will be conducted while the infant is cared for by a familiar laboratory assistant, *after* infant assessment (attention/impulsivity) is completed. By 36-months, infants can be assessed alone and therefore mother and infant assessments can occur simultaneously. Note, that individual research assistants are assigned to specific mother-infant pairs for which they will be responsible for the duration of the study. Our intention here is to allow individual assistants to become familiar with mothers/infants and thus ease the level of apprehension associated with testing.

The measures and procedures described here for the infants occur in parallel with those of the mothers. In this plan, we have considered the joint burden of assessment on mother and infant to ensure that: 1) home rather than laboratory visits predominate in the first year; 2) questionnaires are distributed over time; 3) no session is longer than 2 hours total; 4) the time required for individual infant tasks is within the limits appropriate to the age of assessment and 4) mother-child separations do not occur in the first 18 months, are no more than 30-45 minutes at 18 months, and no more than an hour at 36 months. During mother-infant separations, infants will remain with a familiar assistant. Few of the test materials included in this protocol comprise diagnostic tools. However, where appropriate (e.g. depression scales), subjects will be immediately notified if test scores suggest pathology and offered a referral to an appropriate mental health specialist.

Finally, we mention the possibility of brain imaging in children. However, the details of the procedure have not yet been finalized and would pertain only to the children of 36 months of age. Please note that we will conduct imaging only in unanesthetized subjects. Under no conditions would we consider the possibility of any sedative agent. The Montreal Consortium for Brain Imaging Research, of which Lupien, Pruessner, Meaney, and Paus are members and Evans the Director, is the lead site for the NIH Developmental Brain Mapping project. The lead site for neuroimaging (structural MRI) of younger children is at Washington University in St. Louis. To date, this site has imaged ~150 children using no anesthetic or sedative and is the most experienced site in the world. We have had extensive consultation with this team and the information provided suggested that our original plan to image infants was unlikely to be feasible with the current CIHR budget. In contrast, they report remarkable success with children above 24 months (i.e., >70%), especially when children are prepared for scanning with experience in a Mock Scanner, which simulates the imaging conditions (noise, confinement, etc.) with the exception of the actual

magnets. The Douglas Hospital Research Centre will be purchasing such a scanner over the next 8 months.

General procedure

Six-month home interview: First visit (90 minutes)

Mother

1. Questionnaires (Time required for completion is approximately 90 minutes)

1.1 Stress:

1.1.1. Perceived Stress Scale. Maternal stress measures are assessed at 6 and 12 months postpartum. Perceived stress is assessed using the short form of the Perceived Stress Scale (4 items) (Cohen et al. 1983). The short form has an internal consistency of .72 (coefficient alpha) and two month test-retest reliability of .55.

1.1.2. The Parental Stress Scale, (Berry and Jones, 1995) is an 18-item scale, which has good internal reliability (alpha = .83) and good 6-week test-retest stability (.81). This scale is much shorter than the Parenting Stress Index and is especially useful when samples include parents whose child does not have a developmental disability.

1.1.4 Chronic stressors are assessed via measures of money problems, food security, housing problems, work stress, negative interactions with others and conjugal violence. The money problem questions were drawn from the Daily Hassles Scale (Kanner et al., 1981). Each woman is asked if, since the beginning of their pregnancy, they have lacked money to: (1) pay for housing, (2) pay for electricity, hot water or heating, (3) buy food, (4) buy prescription medications, (5) buy or pay for anything else that she or her family needs.

1.1.5. Work stress is assessed with a 13-item abbreviated version (Fenster et al.,1995) of the Karasek Job Content Questionnaire (Karasek et al., 1986). This measure includes four items on job demands and five items on job control as well as four items on social support at work.

1.1.6.The negative interactions measure asks the first name and relationship of each person with whom the woman can expect to have unpleasant disagreements or to be made angry or upset. The woman also indicates the number of times in the past two weeks that she has had a disagreement with each of these people. This is taken from the Arizona Social Support Interview Schedule (ASSIS) (Barrera M., 1981).

1.1.7. The Marital Strain Scale (Pearlin et al., 1978) is used to assess chronic stress with one's romantic partner. The 9 items represent the three items with the highest factor loadings for each of the three factors contributing to the measure of marital strain. In addition, the Abuse Assessment Screen is used to assess conjugal violence. This five-item instrument assesses the frequency, severity, perpetrator, and body sites of injury (McFarlane et al., 1992; Parker et al., 1993).

1.1.8 Lazarus and Folkman's measure of primary appraisals (of challenge, threat, and centrality) is administered (14 items) (Folkman and Lazarus, 1985).

1.1.9 Reactive responding. This is a 9 items scale that we obtained from Shelley Taylor based on her work with the MacArthur Network on socioeconomic status and health. The idea is that those who have experienced chronic stress develop a hypervigilant response style to ambiguous environmental stimuli.

1.2. Resilience

The three core measures of personal resilience are self-esteem, optimism and beliefs about control. In addition, just world beliefs, life changes, motives for maternal caregiving (maternal care regulation), and commitment (willingness to sacrifice) are assessed.

1.2.1. Personal Resilience. Self-esteem is assessed using the short form (4 items) of the Rosenberg Self-Esteem Scale (Major et al., 1998), the most widely used instrument to assess self esteem (Rosenberg M., 1965; Valliéres and Vallerand, 1990).

1.2.2 Optimism is assessed using a short form of the Life Orientation Test (Scheier and Carver, 1985). The full scale has good internal consistency, (alpha = .76) and good test-retest stability (.79). The short form we are using includes all 8 items from the original scale and deletes the four filler items.

1.2.3 Beliefs of control is assessed using the 7 items mastery beliefs scales (Pearlin and Schooler, 1978).

1.2.4 Life changes. This is an open-ended question at the end of the interview that elicits qualitative data from the woman. "Sometimes after having a baby, people think about life in a different way. In what ways, if any, do you think about life differently now?" The woman's response is tape recorded and transcribed.

1.2.5 Belief in a just world. Theoretical and empirical work has suggested that this represents a core belief that is fundamentally challenged by major negative life events. A prospective study of coping with the loss of a spouse found that a belief in a just world buffered the effects of spousal loss on psychological distress (8 items) (Bonanno et al., 2002).

1.2.6 Maternal care regulation. This is derived from self determination theory (Deci and Ryan, 1991) and corresponds with the work of one of the team members showing that the motivational bases for caregiving was associated with caregiver well-being among those caring for a loved one with Alzheimer's. The idea is to differentiate between caregiving driven by guilt vs. more self determined motives for caregiving (8 items). The latter type should be more nurturing and promote greater autonomy in the child.

1.2.7 Sacrifice scale. These three questions measure the extent to which a woman is having to sacrifice other things for caregiving. It is an indicator of commitment to the caregiving relationship.

1.3. Social resources:

1.3.1 Social support is assessed with five items from the ASSIS (Barrera M., 1981). This assesses private feelings, material aid, advice, positive feedback, and physical assistance. The social participation domain (have fun or relax with) was not assessed. Included are questions assessing the number of people available "in time of need" and to "confide in or talk freely about your problems." It then assesses whether the woman has needed support in the past two weeks in each of the five areas specified above. For each need, the woman is then asked if she received help, and if not, how many times she was left without anyone to help her in this way.

1.3.2. Relationship quality is assessed with the six item Quality of MarriageIndex (Norton R., 1983). The QMI has been found comparable to the Dyadic Adjustment Scale (DAS), correlating with the DAS at .90 (Heyman et al., 1994). Moreover, one of the items in the QMI (and retained in our study) is Magical Question 31 from the DAS. That is the question that correlates with the other 31 items of the 32-item DAS at .70 (Goodwin R., 1992). In addition, the one-item Inclusion of Other in Self Scale is given to

assess the degree of relationship closeness. Finally, the McGill Assessment of Relationship Commitment (Lydon and Gagné) is assessed. In total, 11 questions are administered to assess relationship quality.

1.3.3. Attachment is assessed with items from Brennan and colleagues' comprehensive review and distillation of the most commonly used measures of adult attachment (Brennan et al., 1998). Their analysis of responses from 1,076 respondents generated two subscales representing the two theoretical dimensions of adult attachment: avoidance of closeness and anxiety about relationships. Earlier versions of attachment measures included measures of both fear of closeness and fear of dependency. Because both of these fold into the avoidance of closeness measure, four items loading on this subscale were chosen (2 fear of closeness and 2 fear of dependency items). Because anxiety has stood alone as a measure even in earlier versions, only three items with the highest factor loadings for this subscale were retained. We have successfully used this 7-item measure in a series of three experiments (Bartz and Lydon, 2003).

1.3.4. Parental Bonding Inventory (PBI, Parker et al., 1979) is a standard in retrospective assessment of parental educational style (48 items) (Parker and Lipscombe, 1980).

1.4. Health Behaviors:

Health Behaviors are taken at 6, 12, 24 and 36 months post-partum using a 9 items questionnaire**.** Questions assess cigarette smoking, use alcohol, prescribed medications and non-medicinal drugs ("recreational" or "hard" drugs). Breastfeeding will be assessed at 6 and 12 months.

1.5. Depression:

Maternal depression measures are taken at 6, 12, 24 and 36 months postpartum. The Center for Epidemiological Studies, Depression Scale (CES-D) is a 20-item instrument that has excellent internal consistency (.90) and reasonable test-retest reliability (.54) for a scale that should be sensitive to adverse changes in the respondent's environment (Radloff 1997; Fuhrer & Roullion 1989). For the current project, the scale is being used primarily as a continuous measure of change in depression (from the prenatal interview to the one year post partum interview). Although there are cutoff points for classification purposes, caution is needed to avoid a high false positive rate (Santor et al. 1995). These mood factors are reliable and valid for the post-partum population and show consistency across time, (Fleming et al., 1988,1990). Correlations between the current experience scale (CES) and standardized mood scales (e.g. the MAACL, Zuckerman, 1985 and the BDI, Beck et al.,1961) are highly significant (range .55 to .80; Fleming et al.,1988,1990).

Six-month home interview: Second visit (Time: 60 minutes)

Mother

1. Basal salivary cortisol:

Maternal basal cortisol levels are assessed over the diurnal cycle using salivary samples obtained at 0800, 0830,1000, 1200 (with the sample taken prior to lunch), 1600, 1800 and 2200 hours. The samples will be taken over two consecutive days using salivettes at 6, 12, and 24 months postpartum. The samples, which are stable at room temperature for 3-4 days, will be collected by the subject over the course of their normal daytime activity using portable PalmPilot devices as prompts to ensure accurate sample collection. The subject will be asked to enter the exact time in the recorder. Samples will be collected and processed for radioimmunoassay in Meaney's lab, which has considerable experience with salivary hormone assays (e.g., Lupien et al. 1998, 2002). Time: 2 minutes.

2. Home environment evaluation

Household observations checklist/ household satisfaction index.

The household observations checklist is completed by research assistant immediately following home visit and the household satisfaction index requires research assistant to ask specific questions of mother. Both measures provide a basis for understanding the nature of the home setting. The "household observations checklist" is largely extracted from household assessment guides used in other studies. Responses to Questions 1-5 relate to socio-economic position; responses to Question 6 will be key in relation to subsequent neighborhood level surveys. The literature base for Q1-6 is housing and urban planning.

The "household satisfaction index" derives from epidemiological, health sciences and urban studies research. This literature establishes a link between perceived satisfaction with housing and infant respiratory problems and residents' emotional/affect and behavioural measures. Items given here have "good" Cronbach's alpha (better than 0.65, quite high for this literature) and demonstrated utility in relation to health status, broadly defined. Time: 2 minutes.

Infants

1. Questionnaires

Health of the baby and breastfeeding questionnaire

This 30 items questionnaire will be assessing the health of the infant since his birth and also breastfeeding. For the most part, these are yes/no questions. Time: 7 minutes.

2. Standardized assessment and experimental tasks

Psychomotor Development. The Bayley Scales of infant Development (BSID II, Bayley, 1993) are administered at 6, 12 and 36 months. This 1993 revision of the most widely used infant development assessment consists of three scales: the Mental Scale and Motor Scale assess current level of cognitive, language, personal-social, fine and gross motor development; the Behavior Rating Scale assesses behavior during testing. The BSID II is appropriate for 1 to 42 months of age. Normative data were updated in 1993 and data are available for children with Down syndrome, prematurity and prenatal drug exposure. The assessment is conducted by a trained individual and takes 30 minutes at 6 months and 45 minutes at 12 and 36 months. Training for the Bayley assessment is supervised by Renée Séguin, from the "Laboratoire d'étude du nourrisson" at Université du Québec à Montréal.

3. Mother-infant interactions (behavioral observations)

Videotaped Mother-Infant Interaction. Once questionnaires are completed, the researcher sets up equipment for a 20-minute video of mother-infant interactions and takes baseline salivary samples from mother and baby (see below). During the video, mother and infant are alone in the room. Upon completion of the video, at 20 and 40 minutes after the start of the interaction, the researcher takes the second and then third saliva sample.

Observations: Infants are given the opportunity to feed and be changed prior to the video. Mothers are seated on a comfortable stationary chair/sofa and asked to interact as they would if alone with the infant but to remain within the camera's view and not to feed the infant. Behaviours are later coded from videotape using the BEST system. The coded behaviours on each occasion are adjusted to the age of the infant. For example, at 6 months they include attending to baby (enface, looking over baby), affectionate contact (affectionate burping, stroking, poking or hugging), in proximity to infant (with nose within 2 cm on infant's body), vocalizing to infant (talking or singing to infant), and instrumental activities (instrumental burping,

wiping face, adjusting blanket), and infant activities (hands waving, squirming, feet waving, crying, vocalizations).

Inter-observer reliability is estimated by having two independent observers code 6 to 10 videotapes. In prior studies, percent agreement ranged from 70% to 100% for different behaviors.

4. Salivary cortisol

Basal salivary samples are taken at two time points over the day. Saliva will be collected by swabbing the child's cheek with cotton applicator (Q-tips). Once wetted, the Q-tip is placed in a plastic eppendorf. The eppendorf is then stored at -20°C. Samples are taken at 0800h (AM) and at 1600h (PM), for two consecutive days at 6, 12, and 24 months of age. These samples will be coordinated with the maternal samples using the PalmPilot probes that provide cues for sampling for the mother. Only two samples are taken from the children because of the difficulty posed by daycare sampling. Salivary samples will be analyzed in the Meaney lab using radioimmunoassay procedures that are routine for this group.

- **Six-month laboratory visit (25 minutes)**

Infants

1. Standardized assessment and experimental tasks

1.1. Attention/Impulsivity.

These tasks are laboratory-based experimental tasks designed for infants. Because abilities of infants change dramatically over the first few years, most cannot be used across ages. Instead, we use analogous tasks to tap the same abilities. At 6 and 18 months the infant is in a curtained enclosure, in an infant seat or highchair, or on mother's lap, facing a display of three monitors (Fig. 2). For a given task all or only some of the monitors are used. A peephole in the center allows a camera to film the infant's face so that gaze can be scored from the resulting recording. At 36 months the infant is seated at a low table facing the experimenter.

1.1.1. Habituation of visual attention (6 months). This is one of the few paradigms applicable to assess attention control in very young infants. The basic paradigm involves repeated presentation of a stimulus. With repetition, attention normally decreases and the rate of decrease reflects ability to take in and organize information. The rate of decreased attention as well as patterns of attention (e.g. number of looks in a trial, duration of discrete

looks) are related to later cognitive abilities (see Fagen & Ohr, 2001 for a review). Typically, to ensure that attention decrease is specific to the stimulus (i.e., not a result of fatigue) a novel stimulus or modification of the original is presented at the end of the habituation sequence. Many forms of this paradigm with variation in similarity and dimensions of change for the habituated and novel stimuli are used to study questions about infant cognitive abilities. In the present study, two sets of stimuli are used with all trials appearing on the central monitor. In one, five brightly colored stationary fish appear, all facing in the same direction for 8 trials of 15 second each, with three seconds between trials. On the 9th trial the same fish are presented facing in the opposite direction. The other sequence presents a schematic face that changes orientation (upside down) on the novel trial. Measures are based on change in 1) total looking over trials, number of looks per trial, and duration of longest look on each trial. Time: 10 mins.

1.1.2 Visual Expectation Paradigm, (Haith, Hazan, & Goodman, 1988**,** 6 and 18 months) Learning of context dependent sequences requires development of the frontal structure underlying ability to guide behavior using new information available in the context and to inhibit old responses (Clohessy, Posner and Rothbart, 2001). Visual expectation tasks are a classic measure of this type of learning. In our version of the visual expectation paradigm, a single computer-generated stimulus is presented on one of the three monitors according to predetermined sequences, until the infant orients to it (Interstimulus interval = 600 milliseconds). *Simple sequences* have a repeating pattern in which associations between target locations are unique. Each event reliably predicts the next in the sequence. (e.g., 1-2-1-2-1-2). *Complex sequences* have a repeating pattern in which *some* events reliably predict the next target location but others do not (e.g., 1-2-2-1-2-2-1). Infants as young as 3 months learn to anticipate this latter complex sequence (Canfield & Haith, 1991). The presented stimuli are either colorful looming shapes that alternate between squares and diamonds, or human faces alternating between neutral and angry expressions. Six-month-old infants see sequences that use only the lower two monitors; 18-month-olds see sequences using all three monitors. The measures derived are: 1) the number of correctly anticipated trials; 2) latency to anticipate or respond to an image on correct trials and 3) the difference between the number of correctly anticipated trials in the complex sequence and the simple sequence. Time: 10-15 mins (longer for the older group).

2. Questionnaires

The mother will fill out questionnaires about her child while he/she is undergoing evaluation.

2.1. Temperament.

Although there are laboratory-based observational procedures for assessing infant temperament (e.g., Goldsmith & Rothbart, 1996) these are labour intensive and therefore not as widely used as parent report instruments. The Infant Behavior Questionnaire, Revised Version (IBQ, Garstein and Rothbart, 2003) is completed at 6 and 12 months and the toddler version at 36 months. This is a widely used parent-report measure of infant temperament. The items represent 14 scales but factor analysis reveals three primary factors: a) impulsivity, b) negative affectivity and c) effortful control. We chose this instrument, partly because of interest in impulsivity as a key outcome variable and partly because this measure is well researched and has internal and external validity. The questionnaire is completed by the mother. Time: 15 minutes.

2.2 Childbearing Attitudes questionnaires (CAQ).

The Childbearing Attitudes Questionnaire (CAQ), assessed at 6 and 18 postpartum, is a 76-item scale concerning attitudes toward pregnancy and birth, self-esteem, and other interpersonal relationships. Items include 3-6 items for each of 19 different issues important to infants and childbirth, counterbalanced for direction to minimize response bias. Participants respond to each item on a 7-point scale from disagree strongly (1) to agree strongly (7). The attitude factors for the present study were derived from these items by factor analysis (Ruble et al., 1990). Attitude clusters assess 1) feelings of attachment towards her own infant (attachment), 2) feelings of nurturance towards infants in general (other infants), 3) feelings about caretaking activities, 4) feelings of adequacy in the maternal role (maternal adequacy), 5) feelings towards partner/spouse (relationship with partner) and 6) feelings towards their own mothers (relationship with mother). For the present study, we will use 4 subscales: maternal worries, maternal self-confidence, identification with pregnancy, and feelings about children (20 items total). Time: 7 minutes.

- **<u>Twelve-month home interview: First visit (90 minutes)</u>**

Mother

1. <u>Questionnaires</u>

1.1 Interview: Same as described in the 6 months mother evaluation (See first visit, section 1).

1.2 Experience sampling of Mother-infant interactions (for 7 days)

A computer program has been developed to use personal digital assistants (PDAs, e.g. PalmPilot) to collect experience sampling data from the mother for one week when the baby turns one year of age. The PDA will page the mother three times a day and ask her a series of 10 questions about her most recent interaction with the baby. Questions will ask about their most recent interaction with the baby, about the last time the baby cried and finally, the last time during the day, they were doing something else and the baby wanted their attention. For the evening paging, they will be ask whether they wanted and received social support from their partner and felt understood and cared for by him. Other question will ask about their positive and negative affect at the end of the day. This procedure has been tried successfully for the past 4 months as part of the Montreal New Mothers Study. Time: 5 minutes per paging.

- **Twelve-month home interview: Second visit (50 minutes)**

Mother

1. Baseline salivary cortisol

Procedures described in the 6 months mother evaluation (See second home visit, section 1).

Infants

1. Questionnaires.

1.1 Health of the baby and breastfeeding questionnaire. Time: 7 minutes.

2. Standardized assessment and experimental tasks

Psychomotor Development. The Bayley Scales of infant Development (BSID II, Bayley, 1993) are administered at 12 months. The assessment takes 45 minutes.

3. Baseline salivary cortisol

Procedures described in the 6 months infant evaluation (See second home visit, section 4).

- **Eighteen-month home visit (90 minutes)**

Infants

1. Questionnaires.

1.1 Health of the baby questionnaire*.* Time: 7 minutes.

1.2. Social/Emotional Development.
The Infant-Toddler Social and Emotional Assessment (ITSEA, Briggs-Gowan & Carter, 1998, 18, 24 months) is a parent-report questionnaire, designed as a developmentally and clinically sensitive measure of social-emotional competencies and problems in 1-2 year-olds. It consists of 140 items to evaluate four independent behavioral domains: 1) externalizing, (e.g., high-activity, aggression, defiance, and negative emotional reactivity); 2) internalizing, (e.g., mood regulation problems, social withdrawal and extreme inhibition/shyness); 3) dysregulation (e.g., sleep and eating problems, mood regulation); and 4) maladaptation (e.g., repetitive and stereotypic behaviors). Items are rated on a 3-point scale. Low ITSEA scores are associated with maternal depression and other psychiatric comorbidities (Carter et al, 2001). Low social-emotional competencies are also related to attachment insecurity, poor task mastery and emotion regulation, ineffective coping behaviors (Carter et al, 1999) as well as delayed linguistic development (Irwin et al, 2002). Time to complete: 40 minutes.

2. Behavioral observations

2.1. Mother-Infant Interactions : Home Observation with Q Sort (MBQS 18 months) (Duration ± 45 minutes)

Maternal sensitivity, considered a key quality in establishing secure attachment, is assessed with the Maternal Behaviour Q-set (Pederson et al, 1990). The MBQS yields a single score reflecting the mother's responsivity to infant signals. Two observers are needed because one distracts the mother while the other observes. This allows observation of the mother under conditions of multiple attentional demands. MBQS scores correlate with other maternal sensitivity ratings and measures of attachment (Atkinson et al, 1995, 2000, Moran et al, 1992, Pederson et al, 1990). Meta-analysis (Atkinson et al, 2000b) demonstrated that MBQS scores correlate

with infant attachment security significantly more highly than other observational techniques (r=.48 vs .21).

- **Eighteen-month laboratory visit (90 minutes)**

Infants

1. Questionnaires

The mother will fill out questionnaires about her child while he/she is undergoing evaluation.

1.1. Temperament.
The Early Childhood Behavior Questionnaire, (Rothbart et al., **200?**) is completed at 18 months. Time: 15 minutes.

1.2. Childbearing Attitudes questionnaires (CAQ).
At 18 months, we will use the same 4 subscales of the original questionnaire: maternal worries, maternal self-confidence, identification with pregnancy, and feelings about children (20 items total). Time: 7 minutes.

2.Standardized assessments and experimental tasks

2.1 Attention/impulsivity

2.1.1 The Attention Network Test (ANT, Fan et al., 2002; 18 months) Assesses executive functions based on latency of response to the direction cued by the middle stimulus in a set. Sets are either congruent (all items point in the same direction) or incongruent (the middle item points in a different direction from the others). Although originally designed for adults, this task has been successfully adapted for children (Swanson et al, 2000; Fan et al, 2002). The current project adapts the standard ANT to study infant attention functions by pairing the middle stimulus with an interesting event (an animated videoclip on one of the lower two monitors) presented subsequently in the location cued by the middle stimulus. In the current study, the cues are sets of five looming fish facing left or right on the central monitor. In congruent sets, all fish face the same side and loom in the same direction. In incongruent sets, the middle fish faces and looms in the opposite direction. The middle fish is a reliable cue to the location of the animation clip, and is also colored more brightly than the others to strengthen the association. During each trial, the cue set is presented for 2 seconds at the top monitor of the triangular array. After a 600-millisecond interval, a 3.5 second animated clip appears in one of the lower two

monitors. The locations where the sets of fish prompt and where the animations appear follow a previously determined random sequence. The training session consists of 10 trials, with all cues congruent, facing left or right in a previously determined random sequence. To facilitate initial pairing between the cue and the location of the animated clip, only the middle fish looms during training; the others are motionless. The test session is a previously determined random sequence of 24 trials in which five fish within the set loom, but only the middle one reliably indicates the location of the subsequent animation clip. The difference between latency to anticipate or respond to the animation clips for congruent and incongruent cues is the measure. Time: 15 min.

2.1.2. Visual Expectation Paradigm, (Haith, Hazan, & Goodman, 1988, 18 months). Time: 15 minutes.

3. Behavioral observations

3.1. Social/Emotional Development.

3.1.1. Attachment (18 months): The *Strange situation* (SS; Ainsworth et al., 1978) is a standard videotaped or filmed laboratory assessment consisting of eight episodes wherein the infant is alone, with mother, with a stranger, or both, in an unfamiliar setting. Episodes are ordered to increase stress in standardized increments that are manageable for the baby. The primary purpose of this paradigm is to assess the establishment and quality of infant-mother attachment, an important early social milestone. Trained coders use standardized coding schemes (Ainsworth et al., 1978; Main & Solomon, 1990) to assign the infant/toddler to one of 4 categories (secure, avoidant, resistant/dependant, disorganized) reflecting the quality of attachment. These categories are differentially associated with later outcomes with the "secure" pattern predicting the most optimal outcomes (e.g., Arend et al, 1979; Renken et al, 1989) and the disorganized pattern having links to later psychopathology (e.g., Carlson, 1998).

In the present study, the *strange situation* serves additional multiple purposes. It 1) is a stressor that reliably elicits a cortisol stress response (e.g., Nachmias et al, 1996; Spangler & Grossmann, 1993; Goldberg et al, in press). For this purpose, it is necessary to administer the *strange situation* in a separate session on its own. Note salivary cortisol samples are taken as described below. 2) allows assessment of social inhibition/impulsitivity by coding infant response to the stranger (Greenberg & Marvin, 1982) and 3) allows coding of maternal behaviours known to disrupt formation of attachment (considered markers of risk for child emotional disturbance) using the Atypical Maternal Behavior Instrument for Assessment and Classification (AMBIANCE, Lyons-Ruth & Block, 1996; Lyons-Ruth et al, 1999). The AMBIANCE will also be applied to other videotaped mother-infant interaction sequences. Time: 60 minutes.

3.1.2. Social Inhibition/Impulsivity: As noted above (3.1.1) behaviour to the stranger in the *strange situation* is coded from video.

4. Cortisol Stress reactivity

Saliva samples are collected from infants for cortisol assays at baseline (arrival), and 20 and 40 minutes after the end of the designated stressor. At 18 months, the stressor is the *Strange situation*.

- **Twenty-four month home visit (50 minutes)**

Mother

1. Questionnaires

1.1 Interview

Short version of the questionnaire described in the 6 months mother evaluation (See first home visit, section 1). We will assess the sources of stress in the mother's life, marital quality and health. We expect the interview to take about 45 minutes to complete**.**

2. Baseline salivary cortisol

Procedures described in the 6 months mother evaluation (See second home visit, section 1).

Infants

1. Questionnaires

1.1. Health of the baby questionnaire. Time: 5 minutes.

2. Baseline salivary cortisol

Procedures described in the 6 months infant evaluation (See second home visit, section 4).

- **Thirty-six month home interview (50 minutes)**

  Infants

  1. Questionnaires.

  1.1 Health of the baby questionnaire*.* Time: 5 minutes.

  2. Standardized assessments and experimental tasks

  2.1 Psychomotor Development. The Bayley Scales of Infant Development (BSID II, Bayley, 1993) are administered at 36 months. The assessment takes 45 minutes.

- **Thirty-six month laboratory visit (90 minutes)**

  Infants

  1. Standardized assessments and experimental tasks

  1.1. Attention/Impulsivity.

  1.1.1. Snack Delay (Goldsmith & Rothbart, 1996, 36 months): After placing a single Cheerio or M&M under a clear plastic cup, the experimenter instructs the child to wait for the bell to ring before lifting the glass and eating the snack. Four trials follow with timed durations of 10, 20, 30 and 15seconds. Halfway through each timed period, the experimenter lifts but does not ring the bell. Children hear the instructions again following trials 1-3. Score (from film or videotape) is the length of time the child is able to delay before lifting the glass. Time: 5 min.

  1.1.2. Whisper Task (Goldsmith & Rothbart, 1996, 36 months) The experimenter instructs the child as follows: 'Let's try to talk very very quietly, just like I am - can you whisper your name to me? OK, let's play this whisper game I know. I have some pictures of some cartoon characters - let's see if you can tell me their names (it's okay if you don't know, it's just for fun). Remember to whisper because that is how you play the game." Cards are presented in fixed order, the experimenter asks for a color label or a description of what the character is doing. A reminder to whisper occurs after the first five cards. Scored from film or videotape, based on number of times child speaks above a whisper. Time: 15 min.

1.1.3. Tower Task (Goldsmith & Rothbart, 1996, 36 months). The experimenter explains how to take turns while building a tower out of wooden alphabet blocks. Children are encouraged to keep adding blocks until the tower falls over. Three towers are built. Coded from videotape, based on the number of times the child violates the rule of "turns." Time: 10 min.

2. Behavioral observations

2.1. Social/ Emotional Development.

2.1.1. Attachment (36-months): Reunion behaviour. The *strange situation* was originally designed for 12-18 month-old infants. Adaptations have been developed for 2-5 year-old children based on evidence (Ainsworth et al, 1978) that the key information is obtained in reunion episodes. At this **assessment, mother and infant are separated during the testing period (approximately 1h**). The mother then returns to the child's testing room and the reunion and free play following it are coded using the scheme for preschool attachment (Cassidy & Marvin, 1987).

2.1.2. Social Inhibition/Impulsivity: As noted above (2.1.1, in the 18-month *Strange situation* section) **behaviour to the stranger in the *strange situation* is coded from video. At 36 months, response to the experimenter is coded using the same coding scheme. In addition, at 36 months, latency to speak to the experimenter and to engage in each task is coded as additional measures of social inhibition.**

3. Cortisol Stress reactivity

Saliva samples are collected from infants for cortisol assays at baseline (arrival), and 20 and 40 minutes after the end of the designated stressor. At 36 months, the stressor is the separation of mother and child during testing.

4. Questionnaire

The mother will fill out the questionnaire about her child while he/she is undergoing evaluation.

4.1. Temperament.

The Early Childhood Behavior Questionnaire (Rothbart et al., **200?**) is completed at 36 months. Time: 15 minutes.

IV. Statistical analysis

Power analysis for maternal sensitivity/infant cortisol response.

We hypothesize that decreased maternal sensitivity will be associated with increased cortisol stress responses (obtained with salivary cortisol measures) in infants in the strange situation test at both 18 and 36 months of age. Published work on *infant's* attachment and cortisol stress responses shows strong effects sizes (consistently significant differences despite small sample sizes). However, we propose to address the influence of *mothers'* behaviour on infants' cortisol stress response. Because infants' behaviour may be more strongly related to their own cortisol stress response than would be the mother's behaviour, we reduced expected effect size to the moderate range (r = .30; Cohen, 1988). We based our power analysis on two continuous variables (maternal sensitivity and net area under the curve, $?_2$ = .008 and a .80 probability of rejecting H_0 if it is actually false. Under these conditions, we need a sample size of 130 (Cohen, 1988). Assuming that 13% of participant subjects do not complete the study (based on data from a previous project drawing on the same recruitment source and requiring similar levels of commitment), we need 145 mother-infant dyads to assess the influence of maternal sensitivity of infant cortisol stress response. Hence the sample size of 240 subjects should be sufficient.

We identified measures that are indicators of stress and potential mediators and moderators of the effects stress on well-being. Most measures are shortened versions of well -documented and widely used measures in the literature. A few are measures that members of the team have obtained from colleagues who are leading researchers internationally. For example, the reactive responding measure was developed by Shelley Taylor of UCLA in collaboration with the MacArthur Network on the study of socioeconomic status and health.

The secondary level of analyses will involve a preliminary structural equation modeling, but please note that this is intended as a "value added" approach to the primary analyses described above that are derived from specific hypotheses. Quantitatively, as was noted in the review, we face a challenge, especially concerning the issue of moderating effects vis-a-vis structural equation modeling. We will draw on the expertise of statisticians from the Montreal Prematurity Team.

The analyses will be performed in two stages: first regression and analysis of variance type models and in the second stage structural equation

modeling. Regression analyses and ANOVA analyses will be used to test the stated hypotheses. In particular, tests of interaction effects will be used to assess effect modification (moderating variables). Longitudinal analyses to assess chronicity of effects will be done using multilevel regression models to take into account intra-subject correlation.

In the second stage, structural equation modeling (SEM) will be used to test a structural model expressing the nature and strength of each link between pairs of constructs, integrated in a comprehensive and detailed model that can be statistically tested for fit. In order to identify the "best" model, a sequence of nested comprehensive models will be formulated. Each will be separately analyzed by SEM. The formulation of each model will be based on the conceptual framework presented in the proposal and will be complemented by the results of the statistical analyses of the first stage. Each model is assessed according to well-known statistical criteria pertaining to (1) the overall statistical fit, (2) tests of significance relative to each link, (3) estimation of direct and indirect effects in the network of causal pathways, and (4) the residuals. A comparison of competing models is possible within SEM, provided these models are nested. Hence, among similar (nested) models that are equally meaningful, the most parsimonious can be identified and is considered as "best". Our hypotheses are essentially based on mediating and moderating variables and causal pathways, rather than simple associations

No standard procedure or formulae are available to estimate sample sizes and power for structural models. Recent simulation studies[140-141]indicate that a sample size larger than 200 will reduce the chances of nonconvergence (of the estimation algorithm), of improper solutions (illegal estimations such as a negative variance) and of unacceptable bias of estimators. The size of the available sample will likely range from 210 (all time points and cortisol) to 292 (all time points no cortisol) to 366 (6 month data) to 1 year only (440) and hence will prove sufficient to meet the preceding guidelines. As recommended by Boomsma and Hoogland, newly developed corrective robust statistics will be used to correct the effect of possible nonnormality of our data.

Nevertheless, please note that the primary statistical analyses are designed to test specific hypotheses concerning infant outcome measures that are assessed using laboratory-based measures such as the Rothbart tests for cognitive testing, behavior in the strange situation, salivary cortisol responses to the strange situation as well as basal cortisol levels, etc. In the literature, the sample sizes for studies of such outcomes rarely exceed 10-12 per group and yield significant effects of maternal sensitivity.

V. Continuing Review process

The principal investigator of the present research project along with the research team will continue to read the scientific literature related to the different variables studied in the present protocol. If there are new data showing that some of the protocols used are harmful for participants, or if one of the variables studied has been shown to be related to negative outcomes, the principal investigator will immediately contact the Research Ethics Committee of Douglas Hospital in order to submit these new issues to the committee. Please note there are 15 investigators associated with this project, each of whom is a leading international expert in their field of study. This level of expertise ensures the use of state-of-the-art testing procedures as well as an awareness of emerging ethical issues with their area of research. All procedures are reviewed on a regular basis by an Executive Committee comprised of Drs. Meaney, Matthews, Lupien, Atkinson, Sokolowski and Lydon. The Executive meets every 2nd month, while members of the project meet twice annually. The topics of ethics are reviewed at each meeting in order to verify adherence to approved procedures and to up-date protocols where necessary.

VI. Literature

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