Einfluss pränataler Stressbelastung auf das Kind

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Liste der wissenschaftlichen Veröffentlichungen zur Inauguraldissertation:

„Einfluss pränataler Stressbelastung auf das Kind“

I) Rothenberger, S. E., Moehler, E., Reck, C., & Resch, F. (2010). Prenatal stress: development of different stress measures over the course of pregnancy” *Psychopathology*. akzeptiert am 13.07.2010.

II) Rothenberger, S. E., Haeussler, S., Resch, F. & Moehler, E. (under revision). Prenatal Stress and Human Infant Development. *Child and Adolescent Mental Health*. eingereicht am 27.01.2010.

III) Rothenberger, S. E., Resch, F., Doszpod, N., Moehler, E. (under revision). Prenatal Stress and Infant Emotional Reactivity at five months of age. *Early Human Development*. 2. Revision eingereicht am 22.07.2010

1. Einführung

Hektik und Stress prägen für viele Menschen den Alltag. Aber was passiert, wenn frau schwanger wird? Hört der Stress während der Schwangerschaft auf oder werden Faktoren zum Stressor, die vorher keine waren? Und wie wird das ungeborene Kind dadurch beeinflusst?

Diese Fragen sind von besonderer Bedeutung, da etwa 90% aller Frauen mindestens einmal in ihrem Leben schwanger werden und die Schwangerschaft eine breite Varianz emotionaler Zustände bei der Mutter hervorrufen kann. Häufig wird die Schwangerschaft an sich schon als Stressor wahrgenommen. Die schwangere Frau stellt darüber hinaus die Umgebung für das werdende Baby dar, so dass angenommen werden kann, dass psychische Zustände der Mutter während der Schwangerschaft Einfluss auf den Fötus haben und langanhaltend zu Veränderungen beim Baby führen können (Ferreira, 1965). Angst, Ärger, Stress und andere Gemütsregungen haben Einfluss auf das Ungeborene während der Schwangerschaft und beeinflussen die weitere Entwicklung des Kindes.

In der Forschung ist der Stress während der Schwangerschaft von besonderer Bedeutung, da negative Auswirkungen dieses Stresses in vielen Studien nicht nur auf die Mutter und auf verschiedene Geburtsparameter, sondern auch auf die weitere Entwicklung der Kinder nachgewiesen werden konnten (Davis & Sandman, 2010; Huizink, 2000; Huizink, de Medina, Mulder, Visser, & Buitelaar, 2002; O'Donnell, O'Connor, & Glover, 2009; Paarlberg, Vingerhoets, Passchier, Dekker, & Van Geijn, 1995; Pagel, Smilkstein, Regen, & Montano, 1990; B. Van den Bergh, 1990; B. R. Van den Bergh & Marcoen, 2004; B. R. Van den Bergh, Van Calster, Smits, Van Huffel, & Lagae, 2008; Wadhwa, Sandman, Porto, Dunkel-Schetter, & Garite, 1993). In einigen Längsschnittstudien konnte belegt werden, dass die Stressbelastung der Mutter in der Schwangerschaft sogar die Entwicklung der Kinder bis ins junge Erwachsenenalter beeinflussen kann (B. R. Van den Bergh, Mennes, et al., 2005; B. R. Van den Bergh, Van Calster, Smits, et al., 2008).

Betrachtet man Studien, die den Zusammenhang zwischen Stressbelastung der Mutter während der Schwangerschaft und der weiteren Entwicklung ihrer Kinder untersuchen, fallen die heterogenen Ergebnisse auf, deren Ursachen in der sehr unterschiedlichen Definition und Operationalisierung des Begriffs „Stress“, aber auch in Mängel bei der methodischen Herangehensweise liegen. Während in Tierstudien Stress kontrolliert appliziert werden kann, weisen Humanstudie häufig aus verschiedenen methodischen, ökonomischen und zeitlichen Gründen Mängel auf. Beispielsweise wurde häufig die Stressbelastung der Mutter während

der Schwangerschaft retrospektiv nach der Geburt der Kinder (u.a. (Mohler, Parzer, Brunner, Wiebel, & Resch, 2006) erfasst. In Deutschland wurde bisher nur eine prospektive Längsschnittstudie durchgeführt (Rieger, 2005). Deshalb erschien es besonders wichtig, im Rahmen dieser Dissertation eine große prospektive Längsschnittstudie in Deutschland zu initiieren, innerhalb derer schwangere Frauen in allen drei Schwangerschaftsdritteln auf aktuelle Stressbelastung mithilfe subjektiven Befragungen und hormoneller Kortisolmessung untersucht werden sollten. Die prospektive Längsschnittstudie, die „Pränatalstudie“ genannt wird, wurde an der Klinik für Kinder- und Jugendpsychiatrie der Universität Heidelberg von November 2007 bis Januar 2010 durchgeführt. Das vorliegende Promotionsprojekt stellt Daten aus verschiedenen Phasen dieser Längsschnittstudie vor: der Pränatal- und Postpartalphase. Im Rahmen dieser prospektiven Längsschnittstudie wurden 108 schwangere Frauen über die gesamte Schwangerschaft- in jedem Schwangerschaftsdrittel- auf aktuelle Stressbelastung mithilfe von verschiedenen Fragebögen zu Depressivität, wahrgenommener Stressbelastung, kritischen Lebensereignissen, Partnerschaft und schwangerschaftsbezogenen Ängsten und mithilfe von Speichelkortisol erfasst. Nach der Geburt der Kinder konnten n = 104 Mutter-Kind-Paare im Alter von fünf Monaten postpartal eingeladen werden, wobei die Kinder auf motorische und kognitive Entwicklung mittels den Bayley Scales of Infant Development II (N. Bayley, 1993) getestet wurden. Ferner wurde die Kagan-Batterie, zur Messung eines Prädiktors für Angststörungen, der „affektiven Reaktivität“, angewandt, welche die affektive Reaktion der Kinder (Schreien, Weinen und Quengeln) auf neue akustische, olfaktorische und visuelle Reize im Alter von fünf Monaten nach Kagan & Snidman (Kagan & Snidman, 1991) erfasste.

Das vorliegende Promotionsprojekt betrachtet zum einen die Ergebnisse zum Zusammenhang zwischen subjektiver Stressbelastung der Mutter, die mithilfe von Fragebögen erfasst wurde und dem pränatalen Kortisolspiegel (**Schrift I**) in jedem Schwangerschaftsdrittel. Zum anderen wurde der Einfluss pränataler Stressbelastung im Kortisolspiegel der Mutter auf die kindliche motorische und kognitive Entwicklung (**Schrift II**) betrachtet. In **Schrift III** wurden die Korrelationen zwischen pränataler Stressbelastung der Mutter und kindlicher Reaktivität auf neue Reize im Alter von fünf Monaten betrachtet (**Schrift III**). Mein eigener Beitrag ist im Text jeweils durch Verweis auf die entsprechende Publikation (**Schrift I bis III**) gekennzeichnet.

2. Wie beeinflusst mütterlicher Stress während der Schwangerschaft die kindliche Entwicklung?

Die *„Fetal Programming Hypothese*“, die als erstes von Barker (Barker, 1998; Barker, et al., 1993) aufgestellt wurde, geht davon aus, dass Personen, die sich selbst als gestresst wahrnehmen, einen erhöhten Kortisolspiegel aufweisen. Ist eine Person einem Stressor ausgesetzt, so wird die Hypothalamus-Hypophysen-Nebennieren-Rinden-(HPA)-Achse aktiviert und vermehrt Kortisol ausgeschüttet. Während der Schwangerschaft wird davon ausgegangen, dass Kortisol vermehrt vom mütterlichen Organismus bei Stressbelastung produziert wird und dabei die Plazentaschranke passieren und den Fötus in seiner Entwicklung, vor allem in seiner Gehirnentwicklung negativ beeinflussen kann. In der zweiten Hälfte der Schwangerschaft wird das Enzym 11ß- Hydroxysteroid Dehydrogenase (HSD) Typ II von der Plazenta produziert, das Kortisol in Kortison umwandelt (Krozowski, et al., 1999). Kortison ist nicht toxisch für das kindliche Gehirn, so dass davon ausgegangen wird, dass der kindliche Organismus in der zweiten Hälfte der Schwangerschaft vor mütterlichem Kortisol und dadurch vor mütterlicher Stressbelastung weitgehend geschützt ist. Dennoch sichert der normale Anstieg des mütterlichen Kortisols während der Schwangerschaft und der Rückgang des plazentaren 11ß-HSD II am Ende der Schwangerschaft die ausreichende Versorgung des Fötus mit Kortisol während des dritten Trimesters der Schwangerschaft, da diese Zeit eine wichtige Rolle für die fetale Lungenreifung und für die Vorbereitung des Fötus auf die Geburt spielt (Hacking, Watkins, Fraser, Wolfe, & Nolan, 2001). Da die Plazentaschranke nur teilweise durch das Enzym 11ß-HSD II geschützt ist, sind mütterliche und fetale Kortisolspiegel miteinander korreliert (Gitau, Cameron, Fisk, & Glover, 1998; Gitau, Fisk, & Glover, 2004). Glukokortikoide spielen eine entscheidende Rolle bei der normalen Entwicklung des Gehirns. Da Glukokortikoide leicht die Blut-Hirn-Schwanke passieren können (Zarrow, Philpott, & Denenberg, 1970), scheinen Glukokortikoide einen wichtige Rolle in der Programmierung des Fötus zu spielen (Matthews, 2000; Trejo, Cuchillo, Machin, & Rua, 2000). Moderate Glukokortikoid-Spiegel scheinen sogar positive Effekte auf die Entwicklung des Gehirns und auf die Entwicklung von Verhaltensstörungen der Kinder zu haben (Kapoor, Dunn, Kostaki, Andrews, & Matthews, 2006).

Diese weitverbreitete Hypothese des fetalen Programmierens wurde bisher in Studien noch nicht grundlegend verifiziert und es bestehen heterogene Studienergebnisse bezüglich des Zusammenhangs zwischen subjektiver Stressbelastung der schwangeren Frau und deren hormoneller Reaktion. Wenn aber subjektive Stressbelastungen nicht mit einem erhöhten Kortisolspiegel zusammenhängen, muss die „Fetal Programming Hypothese“ zumindest in Frage

gestellt werden und andere, komplexere Modelle mit anderen Hormonen, z. B. Endorphinen, als mögliche Mediatoren zum Verständnis herangezogen werden, wie mütterliche Belastung beim ungeborenen Kind ankommen und dort dessen Entwicklung grundlegend verändern kann.

Es scheint deshalb besonders wichtig, im Folgenden die aktuelle Studienlage zum Zusammenhang zwischen subjektiven pränatalen Stressfaktoren und physiologischen Stressparametern als Erstes zu betrachten. Dabei wird die erste Studie von Rothenberger et al. (**Schrift I**) vorgestellt. Daraufhin werden aktuelle Forschungsergebnisse zu Auswirkungen pränataler Stressbelastung auf die weitere kindliche Entwicklung und kindliches Temperament dargestellt.

3. Studien zum Zusammenhang zwischen subjektiver Stressbelastung und physiologischen Stressparametern

Studien, die den Zusammenhang zwischen subjektiven und physiologischen Stressmarkern betrachten, sind immer noch sehr selten, uneinheitlich und abhängig von der Art des Stresses und des Timings, wann während der Schwangerschaft Stress erfasst wurde. Ferner unterscheiden sich die Studien in der Art, wie sie hormonelle Indikatoren für mütterliche Stressbelastung erfassen (Glover, O'Connor, & O'Donnell, 2009). Dabei gibt es die Möglichkeit zum einen basales oder stress-induziertes hormonelles Stressniveau wie beispielsweise nach dem Trierer Stress Test (TST) (Rieger, 2005) zu messen. Zum anderen variieren Studien in der Erfassung des Kortisols, je nachdem ob sie Tagesprofile erstellen (Rieger, 2005; B. R. Van den Bergh, Van Calster, Pinna Puissant, & Van Huffel, 2008) oder einen (Davis, et al., 2007; Davis & Sandman, 2010) oder mehrere Messzeitpunkte (Obel, et al., 2005) am Tag erfassen.

Für das *erste Trimester* fand Rieger (Rieger, 2005) keine Zusammenhänge zwischen subjektiver Belastung der Mutter, dem "Stressindex", der sich aus dem "Prenatal Distress Questionnaire" (Yali & Lobel, 1999), dem "Trierer Inventar zur Erfassung von chronischem Stress" (Schulz & Scholtz, 1999) und der "Perceived Stress Scale" (Cohen, Kamarck, & Mermelstein, 1983) zusammensetzte und dem Kortisolspiegel im Speichel. Pluess, Bolten et al. (Pluess, Bolten, Pirke, & Hellhammer, 2010) dagegen fanden negative Zusammenhänge zwischen mütterlicher Trait-Ängstlichkeit und Baseline Kortisol zum Zeitpunkt des Erwachens und 30min später (Pluess, et al., 2010).

Für das *zweite Trimester* fanden Wadhwa und Kollegen erhöhte Speichelkortisolwerte bei gestressten Frauen im Vergleich zu nicht-gestressten Frauen in der 28. SSW (Wadhwa, Dunkel-Schetter, Chicz-DeMet, Porto, & Sandman, 1996). Obel et al. (Obel, et al., 2005) berichteten einen Zusammenhang zwischen Sorgen über Schwangerschaftskomplikationen und kritischen Lebensereignissen und abendlichem Kortsiolspiegel. Frauen, die sich Sorgen um ihre Schwangerschaft machten, hatte ein 27% höheres Abendkortisol-Niveau. Obel et al. argumentierten, dass das Abendkortisol aufgrund des zirkadianen Rhythmus des Kortisols nicht von Deckeneffekten wie das Morgenkortisol beeinflusst ist, und sie deshalb nur Zusammenhänge mit Kortisolspiegel fanden, die sie am Abend erfassten (Obel, et al., 2005).

Im *letzten Trimester* fand Huizink (Huizink, 2000) heraus, dass Morgenkortisol mit psychischem Stress zusammenhing: Angst vor der Geburt und "daily hassles" (alltägliche Belastungen) hingen positiv mit Morgenkortisol zusammen. Konträr dazu fand Rieger (Rieger, 2005) einen negativen Zusammenhang zwischen psychischem Stress der Mutter und morgendlichem Kortisolspielgel. Davis und Sandman (Davis & Sandman, 2010) konnten moderate negative Korrelationen zwischen wahrgenommener Stressbelastung (hier schwangerschaftsbezogene Ängste, Depressionen und Parenting Stress) und Speichelkortisol am Ende der Schwangerschaft herausfinden (36. SSW.).

Die „Fetal Programming Hypothese" geht davon aus, dass die mütterliche Hypothalamus-Hypophysen-Nebennierenrinden(HPA)- Achse während der Schwangerschaft aktiviert ist und dadurch der Kortisolspiegel der Mutter ansteigt, wenn sie einem Stressor ausgesetzt wird. Der vermehrte Kortisolspiegel kann über die Plazentaschranke die fetale Gehirnentwicklung und die weitere Entwicklung des Kindes auch noch nach der Geburt beeinflussen (Kinsella & Monk, 2009). Die „Fetal Programming Hypothese" nimmt also an, dass sich mütterliche Belastung in ihrem Kortisolspiegel niederschlägt. Wie aber die oben beschriebenen Studien zeigen, ist es noch unklar, ob Kortisol ein wichtiger Mediator sein kann, der erklärt, wie die mütterliche emotionale Reaktion das ungeborene Kind und dessen weitere Entwicklung nach der Geburt beeinflussen kann. Vor allem aber die differenzierte Datenlage zu den verschiedenen Zeitpunkten während der Schwangerschaft ist noch unklar. Da aber die Gehirnentwicklung sehr rasant fortschreitet, ist es besonders sinnvoll, alle Schwangerschaftsdrittel getrennt voneinander zu betrachten.

Aus diesen Gründen hatte die von uns durchgeführte Studie (**Schrift I**) das Ziel, den Zusammenhang zwischen mütterlicher „subjektiver" Stressbelastung, die mithilfe verschiedener Fragebögen erfasst wurde, und basalem Speichelkortisol in jedem Schwangerschaftsdrittel zu untersuchen. Die subjektive Stressbelastung wurde mithilfe des Perceived Stress

Questionniare (PSQ (Levenstein, et al., 1993), dem Pregnancy-related Anxiety Questioannire (PRAQ (Huizink, 2000), dem Fragebogen zur Partnerschaftsdiagnostik (FPD (Hahlweg, 1996) und der Erfragung kritischer Lebensereignisse erfasst. Die physiologische Stressreaktion der Mutter wurde mittels basalem Speichelkortisol gemessen. Die Frauen wurden dazu angehalten, sich an drei hintereinander folgenden Tagen Speichel aus der Mundhöhle in einer ruhigen Situation zwischen 11 und 13 Uhr zu entnehmen. Das Speichelkortisol wurde zu derselben Zeit während der Schwangerschaft erhoben wie die Fragebögen. Es zeigten sich marginal (p <.10) signifikante Korrelationen zwischen dem Perceived Stress Questionnaire (PSQ) von Levenstein und Kollegen (Levenstein, et al., 1993) und des Kortisolspiegels im ersten und zweiten Schwangerschaftsdrittel, nicht aber am Ende der Schwangerschaft. Kritische Lebensereignisse, wie Tod eines nahen Angehörigen, Umzug und Hochzeit der Mutter waren nur zu Beginn der Schwangerschaft positiv mit einem erhöhtem Kortisolspiegel korreliert (p <. 01). Zusammenfassend lässt sich sagen, dass lediglich in der ersten Hälfte der Schwangerschaft ein Zusammenhang zwischen Selbsteinschätzung der Mutter und ihrer hormonellen Antwort bestand.

Wurde die Gruppe der Frauen anhand des 25%-, 50%- und 75%-Perzentils ihres Kortisolspiegels in drei Gruppen von wenig, mittlerer und hoher Stressbelastung eingeteilt (**Schrift I**), zeigten sich lediglich im ersten Schwangerschaftsdrittel signifikante Unterschiede im Perceived Stress Questionnaire (PSQ) von Levenstein et al. (Levenstein, et al., 1993). Frauen, die einen extrem hohen Kortisolspiegel (≥75%) zu Anfang der Schwangerschaft aufzeigten, gaben im PSQ eine höhere wahrgenommene Stressbelastung an als Frauen, die mittlere oder niedrigere Kortisolspiegel aufwiesen. Zu Beginn der Schwangerschaft wurden die Unterschiede der drei Kortisolgruppen im Pregnancy-related Anxiety Questionnaire (PRAQ) von Huizink (Huizink, 2000) marginal signifikant (p <.10). Frauen, die zu Anfang ihrer Schwangerschaft hohe Kortisolspiegel aufzeigten, waren ängstlichster als Frauen mit mittleren und niedrigen Kortisolspiegeln. Im 2. und 3. Schwangerschaftsdrittel wurden diese Unterschiede nicht mehr signifikant. In der Studie von Rothenberger et al. konnte gezeigt werden, dass subjektive Belastung der schwangeren Mutter zu einem erhöhten Kortisolspiegel in der ersten Hälfte der Schwangerschaft führt. Dieses Ergebnis lässt sich sehr gut mit der „Fetal Programming Hypothese“ vereinen, die davon ausgeht, dass der Fötus in der zweiten Hälfte der Schwangerschaft vor mütterlichem Kortisol durch das Enzym 11ß- HSD II geschützt ist. Dennoch bleibt unklar, warum die subjektive Belastung nur in den ersten beiden Schwangerschaftsdritteln eine hormonelle Entsprechung im erhöhten Kortisolspiegel der Mutter zeigte, was für eine „sensible Periode“ in der ersten Hälfte der Schwangerschaft sprechen würde.

Man kann spekulieren, dass im letzten Drittel der Schwangerschaft der Kortisolspiegel so weit erhöht ist, dass es für den schwangeren Organismus nicht mehr möglich ist, auf weitere Belastung mit Kortisolausschüttung zu reagieren. Diese Vermutung würde für einen „Deckeneffekt“ des mütterlichen Kortisolspiegels am Ende der Schwangerschaft sprechen. Die Hypothese eines Deckeneffekts kann mit unseren Daten einer gesunden community-based Stichprobe nicht verifiziert werden. Dies müsste mit einer psychiatrisch erkrankten oder extrem belastenden Gruppe betrachtet werden, da in diesen Gruppen die Varianz mütterlicher Belastung größer ist und auch deren Extremformen häufiger auftreten können.

Nachdem wir nun die pränatalen Zusammenhänge betrachtet haben, sind wir nun an Zusammenhängen pränataler subjektiver und physiologischer Stressbelastung der Mutter und deren Einfluss auf ihr Kind und dessen spätere Entwicklung interessiert. Dafür werden zu Anfang Tier- und Humanstudien und daraufhin die Ergebnisse der zweiten Untersuchung von Rothenberger et al. (**Schrift II**) vorgestellt.

4. Studien zur Auswirkungen pränatalen Stresserlebens auf kindliche Entwicklung

4.1 Tierstudien

Wesentliche Ergebnisse, die den Einfluss pränatalen Stresses der Mutter auf die Entwicklung ihrer Kinder erklären, stammen aus Tierexperimenten. Tierstudien haben den Vorteil, Stress als unabhängige Variable kontrollieren zu können, indem sie Tiermütter einem bestimmten Stressor in der Schwangerschaft aussetzen (z. B. Elektroschocks, Lärm, Separierung der schwangeren Tiere von der Herde, etc.). Danach können sie Outcome- Variablen wie beispielsweise Geburtsgröße, Geburtsgewicht, Behinderungen und behaviorale und physiologische Antworten der Nachkommen unter kontrollierten und standardisierten Bedingungen beobachten. Tierstudien zeigen Auswirkungen des mütterlichen, pränatalen Stresses auf die mütterliche Kortisolausschüttung und auf die Aktivität der Hypothalamus- Hypophysen-Nebennierenrinden-Achse des Fötus, u. a. bei Ratten (Henry, Kabbaj, Simon, Le Moal, & Maccari, 1994). In manchen Studien wurde ein geringeres Geburtsgewicht mit pränatalem Stress assoziiert (Weinstock, Fride, & Hertzberg, 1988). Durch die frühe Kortisolbelastung des fötalen Gehirns wird dieses nachteilig geprägt, was wiederum im weiteren Verlauf verschiedene Probleme in der Entwicklung der Nachkommen nach sich ziehen kann (Schneider, 1992a, 1992b). Eine veränderte Rezeptorendichte im Hippocampus (Amiel-Tison, et al.,

2004; McCormick, Smythe, Sharma, & Meaney, 1995) als auch eine veränderte Funktion der Amygdala (Becker, Abraham, Kindler, Helmeke, & Braun, 2007; Kim, Lee, Han, & Packard, 2001) wurden von einigen Autoren als Ursache für die veränderte Entwicklung beim Fötus und Kind angenommen. Braun und Mitarbeiter haben schwangere Ratten untersucht und fanden bei Nachkommen von Ratten, die während der Schwangerschaft gestresst wurden, weniger Nervenverbindungen im cingulären und orbitofrontalen Kortex heraus (Braun, 2006). Ferner stellten sie fest, dass verschiedene Nervenzellen veränderte Nervenverzweigungen aufzeigten, mit unterschiedlichen Effekten bei weiblichen und männlichen Nachkommen. Im Hippocampus entwickelten männliche Nachkommen kürzere Nervenverbindungen, während dies bei den weiblichen Nachkommen gestresster Ratten nicht auftrat (Braun, 2006).

Huizink et al. (Huizink, Mulder, & Buitelaar, 2004) fassten in ihrer Arbeit Ergebnisse aus Tierstudien zusammen und zeigten große Unterschiede zwischen den Studien in Frequenz, Intensität, Art und Dauer des Stresses, welche Tierart oder welche weiteren Faktoren untersucht und kontrolliert wurden. Deshalb muss man auch bei Tierstudien sehr genau betrachten, welche Art von Stress und welche Outcome- Variablen betrachtet werden. Zusammenfassend zeigt sich, dass pränataler Stress Entwicklungsverzögerungen bei Nagetieren und Primaten hervorrufen kann. Bei Nagetieren ist pränataler Stress mit verringerten explorativen Verhalten, verringerter Emotionalität und beeinträchtigter Anpassung an Konflikt- oder Aversionsbedingungen assoziiert. Exploratives Verhalten in einer Stresssituation oder bei neuen Reizen ist auch bei Primaten durch pränatalen Stress beeinflusst. Lerndefizite konnten in pränatal gestressten Ratten gefunden werden (Archer & Blackman, 1971; Smith, Wills, & Naylor, 1981). Die Studienlage zeigt, dass sich behaviorale Veränderungen bei den Nachkommen nicht auf einen speziellen, scharf umgrenzten Stressor des schwangeren Muttertieres beschränken. Ein pränataler Stressor kann verschiedene Effekte bei unterschiedlichen Tierarten oder auch nur bei bestimmten Stämmen einer Tierart haben. Diese allgemeinen Schlussfolgerungen aus Tierstudien sind von großer Bedeutung, wenn man Pränatalstress bei Menschen untersuchen möchte, da die Ergebnisse aus Tierstudien Schlussfolgerungen für das Verständnis bei der Entwicklung von Psychopathologien im Humanbereich zulassen.

4.2 Humanstudien

Studien, die die Entwicklung der fetalen Hypophysen-Hypothalamus-Nebennierenrinden-Achse (HPA) und die spätere Entwicklung des Kindes von gestressten Müttern betrachten, sind immer noch selten und haben einige methodische Schwächen

(Egliston, McMahon, & Austin, 2007). Methodische Schwierigkeiten erschweren dabei die Interpretation und die Vergleichbarkeit der Studienergebnisse. In Humanstudien können schwangere Frauen nicht wie in Tierstudien, verschiedenen Stressoren ausgesetzt werden. Schwangerschaft an sich wurde häufig schon als relevanter Stressor genannt, vor allem die Ängste und Sorgen, die sich eine Schwangere während ihrer Schwangerschaft macht (Bjelica & Kapor-Stanulovic, 2004).

Es wurden dennoch verschiedene Herangehensweisen genutzt, um Stress bei Menschen dennoch erheben zu können. Eine häufig genutzte Strategie, um Stress während der Schwangerschaft bei Frauen zu untersuchen, ist die Untersuchung schwangerer Frauen bei Naturkatastrophen oder anderen kritischen Ereignissen wie beispielsweise Zweiter Weltkrieg, Terroranschlag am 11.09.2001 oder Wirbelsturm Katharina. Vorteil dieses Vorgehens ist, dass die Vergleichbarkeit des Stressors gegeben ist: alle Frauen haben dasselbe Erlebnis miterlebt. Yehuda et al. (Yehuda, Bell, Bierer, & Schmeidler, 2008; Yehuda, et al., 2009) haben Kinder von Holocaust-Überlebenden betrachtet. Hier konnte festgestellt werden, dass Frauen, die den Holocaust überlebten und eine Posttraumatische Belastungsstörung (PTBS) entwickelten, Kinder hatten, die weniger Cortisol ausschütteten als Kinder von Frauen, die keine PTSB entwickelten nach dem Überleben des Holocausts. Dieser s. g. „Hypokortisolismus“ wird im Zusammenhang mit PTBS häufig in Zusammenhang gebracht und wird durch einen chronischen Belastungszustand und eine Reduktion der Rezeptoren im Kortex der Betroffenen erklärt.

Weitere Möglichkeiten zur Erfassung pränatalen Stresses beinhalten die Erfassung von s. g. „objektiven“ Stressparametern. Darunter versteht man unter anderem kritische Lebensereignisse wie Tod eines Angehörigen, Umzug, Hochzeit, etc. (Bergman, Sarkar, O'Connor, Modi, & Glover, 2007; Cote-Arsenault, 2007; Glynn, Schetter, Wadhwa, & Sandman, 2004; Lobel, et al., 2008; Zhu, Tao, Hao, Sun, & Jiang, 2010) oder medizinische Risikofaktoren wie Alkoholkonsum, Rauchen oder Drogenmissbrauch (Garcia-Algar, Puig, Vall, Pacifici, & Pichini, 2004; Huizink, 2009; Huizink & Mulder, 2006; Mohler, et al., 2008; Ornoy & Ergaz, 2010; Pichini & Garcia-Algar, 2006; Stroud, et al., 2009). Diese verschiedenen Risikofaktoren wurden häufig im Zusammenhang mit geringerem Geburtsgewicht (Paarlberg, et al., 1995; Wadhwa, et al., 1993), Frühgeburtlichkeit (Dole, et al., 2003; Pagel, et al., 1990) und geringerem Kopfumfang (Lou, et al., 1994; Lou, et al., 1992) gebracht. Die so genannten „daily hassles“ schauen sich alltägliche Dauerbelastungen an wie ständige Überforderung und Arbeitsbelastung. Tägliche Belastungen der schwangeren Frau gemessen mit der „Everyday Problem List“ hingen mit geringerem kognitiven Entwicklungsstand mit acht Monaten des

Kindes zusammen (Buitelaar, Huizink, Mulder, de Medina, & Visser, 2003; DiPietro, Ghera, Costigan, & Hawkins, 2004).

Eine weitere Möglichkeit zur Erfassung pränataler Stressbelastung ist die Befragung der betroffenen Frauen mittels Fragebögen. Dieses Verfahren nenne ich im weiteren Verlauf dieser Arbeit „subjektive“ Verfahren, da hier die Frauen ganz subjektiv ihre eigene Stressbelastung einschätzen müssen. Selbsteinschätzungsfehler, sowie soziale Erwünschtheit können hier die Antwort beeinflussen. Dennoch wird diese Methode häufig genutzt, da diese am einfachsten und ökonomischsten erfasst werden kann. Dabei kann man bei den Fragebögen unterscheiden, welche Symptome oder Arten des Stresses sie abfragen, wie beispielsweise Fragebögen zur depressiven Symptomatik der Frauen in der Schwangerschaft. Martini et al. (Martini, 2009) berichten einen Prozentsatz von bis zu 15% klinisch relevanter Depressionen während der Schwangerschaft. Wobei Figueiredo & Costa herausfanden, dass mütterliche Depression zu einer geringeren Beteiligung vor der Geburt und Ängstlichkeit zu einer geringeren Beteiligung nach der Geburt führte (Figueiredo & Costa, 2009). In einer Studie von Diego und Mitarbeitern (Diego, et al., 2009) wurde herausgefunden, dass pränatale Depression fetales Wachstum einschränkt. Weitere Studien, die sich mit pränataler Depression beschäftigten, fanden negative Auswirkungen auf Geburtsparameter (Reck, 2009) und kindliche Entwicklung heraus (Davis, et al., 2007; Davis & Sandman, 2010; Dipietro, Costigan, & Sipsma, 2008; O'Connor, Heron, Golding, Beveridge, & Glover, 2002).

Studien, die sich mit Ängstlichkeit beschäftigten, unterschieden sich in der Untersuchung der State-Ängstlichkeit (O'Connor, et al., 2002; O'Connor, Heron, Golding, & Glover, 2003; Reck, 2009; B. Van den Bergh, 1990; B. R. Van den Bergh, Mennes, et al., 2005) oder schwangerschaftsbezogener Ängste (Huizink, 2000). Ängste der Mutter sagten kindliche emotionale und behaviorale Probleme bis 33 Monate postpartal voraus (O'Connor, et al., 2002) und zeigten einen negativen Einfluss auf die Aufmerksamkeitsleistung von Jungen im Alter von 15 Jahren (B. R. Van den Bergh, Mennes, et al., 2005). Reck et al. (Reck, 2009) berichteten einen signifikanten Zusammenhang zwischen Ängsten und Depressionen und fanden eine verlängerte Geburt als Konsequenz der pränatalen Ängste heraus. Eine der wenigen Studien, die einen positiven Einfluss pränataler Stressbelastung auf kindliche Entwicklung zeigen konnten, war die Studie von DiPietro et al. (DiPietro, Novak, Costigan, Atella, & Reusing, 2006). Sie berichteten, dass milde Formen von Stressbelastung eventuell einen positiven Einfluss auf kindliche Entwicklung in den ersten zwei Lebensjahren haben können. Sie fanden Zusammenhänge zwischen pränataler Ängstlichkeit, nichtspezifischem Stress, depressiven

Symptomen im zweiten Schwangerschaftstrimenon und einer besseren motorischen Entwicklung im Alter von zwei Jahren (DiPietro, et al., 2006)

In einer Längsschnittstudie (E. P. Brouwers, A. L. van Baar, & V. J. Pop, 2001; E. P. M. Brouwers, A. L. van Baar, & V. J. M. Pop, 2001) über State-/ Trait- Ängstlichkeit während der Schwangerschaft wurde herausgefunden, dass pränatale Ängstlichkeit mit externalisierenden Problemen in der späteren Kindheit zusammenhing: ein hoher pränatales Angstlevel im zweiten Trimenon der Schwangerschaft erklärte 22 % der Varianz der Aufmerksamkeits-/ Hyperaktivitätsstörung (ADHS)-Symptome mit acht und neun Jahren und 15 % der Varianz von externalisierenden Problemen. In der AVON Longitudinal Studie (B. R. Van den Bergh & Marcoen, 2004) verdoppelte pränatale Ängstlichkeit das Risiko für Hyperaktivität, Aufmerksamkeitsprobleme und Verhaltensausauffälligkeiten bei den Kindern mit drei und 12 Wochen bis hin zu 47 und 81 Monaten.

Viele Studien versuchten eine physiologische Reaktion der schwangeren Frauen zu ermitteln. Vor allem Cortisol wurde in den meisten Studien erfasst, weil ihm entsprechend der „Fetal Programming Hypothese“ eine wichtige Rolle im Stresssystem und der Auswirkung mütterlicher Stressbelastung auf die kindliche Entwicklung zugeschrieben wird. Ferner kann Cortisol einfach über Speichelproben erfasst werden. Davis und Sandman fanden einen negativen Einfluss des mütterlichen Kortisolspiegels im ersten Schwangerschaftsdrittel auf die Entwicklung des Kindes im ersten Lebensjahr heraus. Kortisol im letzten Drittel dagegen hatte einen positiven Einfluss auf die kindliche Entwicklung mit 12 Monaten (Davis & Sandman, 2010). Konträr dazu fanden Buitelaar und Kollegen (Buitelaar, et al., 2003) heraus, dass der Kortisolspiegel am Morgen negativ mit motorischer und kognitiver Entwicklung mit drei Monaten und negativ mit motorischer Entwicklung mit 8 Monaten des Kindes zusammenhingen. Diese konträren Forschungsergebnisse des Zusammenhangs zwischen pränatalem Kortisolniveau der Mutter und der kindlichen Entwicklung veranlassten uns, diesen Zusammenhang über alle drei Messzeitpunkte der Schwangerschaft zu betrachten (**Schrift II**). In unserer Untersuchung an n = 108 schwangeren Frauen wurde basales Speichelkortisol an drei hintereinander folgenden Tagen zwischen 11 und 13 Uhr in einer ruhigen Situation zu Hause erhoben. N = 104 Mutter-Kind-Paare wurden im Alter von fünf Monaten in die Klinik für Kinder- und Jugendpsychiatrie eingeladen, um kognitive und motorische Entwicklung mithilfe der Bayley Scales of Infant Development III (N. Bayley, 1993) zu untersuchen. In unserer Studie zeigte sich ein signifikanter negativer Zusammenhang zwischen Kortisolbelastung der Mutter im letzten Schwangerschaftsdrittel und der kindlichen kognitiven Entwicklung im Al-

ter von fünf Monaten. Kinder, deren Mütter mehr Kortisol am Ende ihrer Schwangerschaft ausschütteten, zeigten im Alter von fünf Monaten eine schlechtere mentale Entwicklung.

In einem zweiten methodischen Schritt (**Schrift II**) wurde die Gruppe der Frauen anhand des 25%- und 75%- Perzentil ihres Kortisolspiegels in Extremgruppen mit extrem wenig und hoher hormoneller pränataler Stressbelastung aufgesplittet und die kognitive und motorische Entwicklung ihrer Kinder betrachtet. Lediglich am Ende der Schwangerschaft konnten signifikante Unterschiede in den Extremgruppen im motorischen *und* kognitiven Entwicklungsstand der Kinder gefunden werden. Frauen, die einen extrem niedrigen Kortisolspiegel am Ende der Schwangerschaft aufwiesen (≤ 25%), hatte Kinder, die im Alter von fünf Monaten besser in der motorischen und kognitiven Skala der Bayley Scales (N. Bayley, 1993) abschnitten. Interessanterweise zeigten sich diese Unterschiede der Extremgruppen lediglich am Ende der Schwangerschaft. Bei der zweiten Studie von Rothenberger et al. zeigten sich nun negative Zusammenhänge zwischen pränatalem Kortisolniveau im letzten Schwangerschaftsdrittel und der kindlichen Entwicklung. Davis und Kollegen (Davis & Sandman, 2010) fanden am Ende der Schwangerschaft positive Korrelationen zwischen Kortisolspiegel der Mutter und kindlicher Entwicklung. Buitelaar et al. (Buitelaar, et al., 2003) fanden negative Korrelationen zwischen Morgenkortisol am Ende der Schwangerschaft und motorische und mentale Entwicklung mit drei und acht Monaten des Kindes. Diese konträren Ergebnisse können auf die Erfassung des pränatalen Kortisolspiegels zurückgeführt werden. Davis und Kollegen (Davis & Sandman, 2010) erfassten Kortisol zu einem Messzeitpunkt gegen 14 Uhr, wobei Buitelaar et al. (Buitelaar, et al., 2003) Tagesprofile erstellte und lediglich Zusammenhänge mit Morgenkortisol finden konnte. Die Ergebnisse weisen darauf hin, dass die „Fetal Programming Hypothese“ dahingegen verifiziert werden kann, dass mütterlicher Kortisolspiegel schädlich für die Entwicklung beim Kind ist. Dennoch muss die Annahme, dass über das Enzym 11 ß-HSD II Kortisol in Kortison am Ende der Schwangerschaft umgewandelt wird und der Fötus in dieser Phase der Schwangerschaft vor mütterlichem Kortisol geschützt ist, durch unsere Studienergebnisse zumindest stark in Frage gestellt werden.

Die letzte Studie (**Schrift III**) von Rothenberger et al. beschäftigt sich nun mit der Frage, wie mütterliche subjektive und physiologische Stressbelastung in der Schwangerschaft mit kindlicher Reaktivität zusammenhängt. Kann pränataler Stress kindliches Temperament beeinflussen? Dazu wird anfangs die aktuelle Studienlage zu Auswirkungen pränatalen Stresses auf kindliches Temperament vorgestellt und danach die Ergebnisse der Studie von Rothenberger et al. berichtet.

5. Studien zur Auswirkungen pränatalen Stresserlebens auf das kindliches Temperament

Kindliches Temperament kann zum einen durch mütterliches Urteil wie beispielsweise mithilfe des Infant Behavior Questionnaires (IBQ) (Rothbart, 1981) oder zum anderen durch Verhaltensbeobachtung des Kindes beispielsweise mittels des Kagan-Paradigmas (Kagan & Snidman, 1991) erfasst werden. Das Kagan-Paradigma erfasst die „kindliche Reaktivität" auf neue Reize, ein fundamentales Charakteristikum der Behavioralen Inhibition (BI). Behaviorale Inhibition wird hierbei als angeborenes Trait bezeichnet, auf neue Reize, Situationen, Ereignisse und Menschen mit Angst zu reagieren (Kagan, Reznick, & Gibbons, 1989).

Studien, die sich auf mütterliches Urteil bezogen, konnten zeigen, dass mütterlicher Kortisolspiegel am Ende der Schwangerschaft einen positiven Zusammenhang mit kindlicher negativer Reaktivität auf neue Reize zwei Monate postpartal aufzeigte. Mütterliche subjektive Stressbelastung wie Ängstlichkeit und Depressivität sagten kindliches Temperament voraus (Davis, et al., 2007). Ferner hatten Mütter, die in der 25. SSW. einen geringen Korticotropin-releasing Hormon (CRH)- Spiegel hatten, Kinder, die mit zwei Monaten weniger Distress und Angst im IBQ zeigten (Davis, et al., 2005).

In der Verhaltensbeobachtung nach Kagan & Snidman (Kagan & Snidman, 1991) konnte dieselbe Arbeitsgruppe einen positiven Zusammenhang zwischen pränataler Depressivität und Ängstlichkeit, nicht aber der postpartalen Stressbelastung auf kindliche negative behaviorale Reaktivität auf neue Reize zeigen (Davis, Snidman, et al., 2004). Andere Verhaltensbeobachtungen in verschiedenen Situationen, wie Fütter-, Wickel- oder Badesituationen zeigten einen Zusammenhang mütterlicher pränataler Kortisolbelastung im letzten Schwangerschaftsdrittel und vermehrtem Schreien, Weinen und negativen Gesichtsausdrücken während einer Badesituation in den ersten 20 Lebenstagen (de Weerth, van Hees, & Buitelaar, 2003). DiPietro und Kollegen beobachten kindliche Reaktivität intrauterin und berichteten, dass Föten, die mit erhöhter motorischer Aktivität auf mütterliche Konfrontation mit neuen Reizen reagierten, später auch Kinder waren, die im Alter von sechs Wochen mehr Irritabilität in einem Entwicklungstest zeigten (DiPietro, Ghera, & Costigan, 2008).

Moehler und Kollegen (Mohler, et al., 2006) führten eine Studie durch, bei der sie kindliche Reaktivität nach Kagan & Snidman (Kagan & Snidman, 1991) in einer Laboruntersuchung der Kinder und die mütterliche pränatale Stressbelastung retrospektiv zwei Wochen postpartal mit Fragebögen erfassten. Sie fanden heraus, dass Mütter mit niedrigerem pränatalem Stresslevel häufiger Kinder mit höherer affektiven Reaktivität im Alter von vier und 14 Monaten hatten, die also mehr Schreien, Weinen und Quengeln bei der Präsentation neuer

Reize zeigten (Moehler, et al., 2008). In dieser Längsschnittstudie an über 100 Mutter-Kind-Paaren konnte bei einer weiteren Untersuchungswelle mit 5.5 Jahren der Kinder gezeigt werden, dass die kindliche emotionale Reaktion auf neue Reize (Schreien, Quengeln, Weinen) im Alter von vier und 14 Monaten bei Mädchen, nicht aber bei Jungen mit 5.5 Jahren Schüchternheit und Gehemmtheit voraussagten (Moehler et al., in preparation). Eine Vielzahl von Studien konnte belegen, dass extrem inhibierte Kinder in ihrer späteren Kindheit (Hirshfeld-Becker, et al., 2007; Kagan, Reznick, & Snidman, 1987; Kochanska, Murray, & Coy, 1997) und ihrem Jugendalter mehr Gehemmtheit und Schüchternheit mit Peers zeigten (Schwartz, Snidman, & Kagan, 1999). Ferner wiesen Kinder, die mit 14 Monaten inhibiert waren, mit drei und vier Jahren mehr schüchternes und zurückhaltendes Verhalten, eine höhere Rate von sozialen Ängsten mit 7.5 Jahren und mehr soziale Phobien im Jugendalter auf (Biederman, et al., 1993; Biederman, et al., 1990; Hirshfeld, et al., 1992; Kagan & Snidman, 1991; Rosenbaum, et al., 1992).

Angst oder Inhibition vor unbekannten Ereignissen, Menschen oder Stimuli in der frühen Kindheit kann deshalb als wichtiger Prädiktor für spätere kindliche und jugendliche emotionale Entwicklung angesehen werden. Eine extreme Inhibition der Kinder mit 14 Monaten scheint das Risiko zu erhöhen, eine internalisierende Störung in der späten Kindheit zu entwickeln (Rubin, Burgess, Dwyer, & Hastings, 2003; Rubin, Hastings, Stewart, Henderson, & Chen, 1997). Eine erhöhte affektive Reaktivität, das bedeutet die emotionale Reaktion auf neue Reize, die sich bei Kinder häufig in Weinen oder Quengeln zeigt, stellt einen fundamentalen Trait der behavioralen Inhibition dar (Moehler, et al., 2008) und scheint eine wichtige Rolle für die langfristige emotionale Entwicklung zu spielen.

Im Moment ist das Wissen über den Ursprung dieser frühen und langanhaltenden behavioralen Temperamentsunterschiede sehr eingeschränkt. In der Tierforschung mit Mäusen konnten moderate Zusammenhänge zwischen spezifischen Stellen des Gultamat Acid Decarboxylase Gens und der behavioralen Inhibition gezeigt werden (Smoller, et al., 2001). Ferner konnte dieselbe Arbeitsgruppe zeigen, dass moderate Relationen zwischen einem Allel des Kortiocotropin-Releasing Hormons (CRH) und der behavioralen Inhibition bestehen (Smoller, et al., 2003).

Moehler und Kollegen (Mohler, et al., 2006) waren die Ersten, die einen Zusammenhang zwischen dem Temperamentsmerkmal der behavioralen Inhibition und der pränatalen Stressbelastung der Mutter herausfanden. In dieser Studie wurde die pränatale Stressbelastung retrospektiv zwei Wochen postpartal mittels Fragebögen erfasst weswegen keinerlei hormonelle Variablen während der Schwangerschaft erfasst werden konnten. Durch

die retrospektive Befragung der Frauen nach pränataler Stressbelastung muss kritisch beleuchtet werden, ob die Einschätzung der Mütter nicht durch verschiedene Faktoren beeinflusst sein könnte. Die Geburt als einschneidendes Erlebnis beispielsweise könnte die Wahrnehmung der Belastung während der Schwangerschaft beeinflussen, sowie das kindliche Temperament nach der Geburt. Da dieser Zusammenhang des pränatalen Stresses auf kindliche emotionale Entwicklung sehr bedeutend ist, wurde eine prospektive Studie durchgeführt, die Stress in der Schwangerschaft prospektiv mithilfe von Fragebögen und Kortisolspiegel untersuchte. Diese Folgestudie (**Schrift III**) versuchte, die vorherigen Ergebnisse von Moehler und Kollegen (Mohler, et al., 2006) zu replizieren.

Innerhalb des Promotionsprojekts (**Schrift III**) wurden schwangere Frauen in jedem Schwangerschaftsdrittel auf aktuelle Stressbelastungen mithilfe von Fragebögen und Kortisolmessung untersucht. Im Alter von fünf Monaten wurde die kindliche emotionale Reaktion auf neue visuelle, akustische und olfaktorische Reize mithilfe der Kagan Batterie nach Kagan & Snidman (Kagan & Snidman, 1991) erfasst. Schreien, Quengeln und Weinen bei der 20- sekündigen Konfrontation mit neuen Reizen wurde mit 1 kodiert. Ein Reaktivitäts-Score von 0- 16 konnte erreicht werden. Die subjektive pränatale Stressbelastung wurde mithilfe des Edinburgh Postnatal Depression Questionniare (EPDS (Cox, Holden, & Sagovsky, 1987), des Perceived Stress Questionniare (PSQ (Levenstein, et al., 1993) und der Erfragung kritischer Lebensereignisse ermittelt. Die physiologische Stressreaktion wurde anhand des basalen Speichelkortisolspiegels an drei hintereinander folgenden Tagen zu jedem Schwangerschaftsdrittel erfasst.

In dieser Studie (**Schrift III**) wurde zum einen festgestellt, dass es negative korrelative Zusammenhänge zwischen wahrgenommener Stressbelastung im PSQ und der kindlichen affektiven Reaktivität im zweiten und dritten Trimenon gab. Frauen, die während der zweiten Hälfte der Schwangerschaft weniger Stress bei sich wahrnahmen, hatten Kinder, die im Alter von fünf Monaten vermehrt mit Weinen und Quengeln auf neue Reize reagierten- inhibierter waren. Interessanterweise waren die Zusammenhänge zwischen kindlicher Reaktivität und den anderen pränatalen Stressparametern wie Depressivität und externe Stressfaktoren auch negativ korreliert, diese Zusammenhänge wurden aber nicht signifikant. Auch konnte kein Zusammenhang zwischen pränataler mütterlicher Kortisolbelastung zu keinem der Messzeitpunkte während der Schwangerschaft und kindlicher Reaktivität gezeigt werden.

In einem weiteren methodischen Schritt (**Schrift III**) wurde die gesamte Stichprobe anhand der kindlichen Reaktivität in zwei Extremgruppen eingeteilt. Hierbei wurde ein Cut-off Score von ≥ 7 für die Aufteilung der Gruppe gewählt, die bereits Kagan (Kagan, 1994)

verwandte. Es wurden für die beiden Extremgruppen der extrem inhibierten und der extrem uninhibierten Kinder Gruppenunterschiede mittels Mann Whitney U-Test getestet. Im ersten Schwangerschaftsdrittel fanden sich keine signifikanten Unterschiede dieser beiden Gruppen. Im zweiten Schwangerschaftsdrittel wurden signifikante Unterschiede der beiden Gruppen im Perceived Stress Questionnaire (PSQ) von (Levenstein, et al., 1993) und allen Subskalen des PSQ (Anforderungen, Anspannung, Sorgen, Freude) ersichtlich. Im dritten Schwangerschaftsdrittel konnten nur marginal signifikante Unterschiede der beiden Gruppen im PSQ und den Subskalen Anforderungen, Sorgen, Anspannung, nicht aber in der Subskala Freude gefunden werden. Im zweiten und dritten Schwangerschaftsdritteln zeigten sich die Unterschiede in dieselbe Richtung: Frauen, die weniger Anforderung, Anspannung, Sorgen und mehr Freude in der Schwangerschaft aufwiesen, hatten Kinder, die mehr affektive Reaktivität (Schreien, Weinen und Quengeln) in der Kagan-Batterie zeigten.

6. Fazit

Die drei Studien von Rothenberger et al. beschäftigten sich in einem prospektiven Längsschnittstudiendesign mit Stress während der Schwangerschaft und dessen Auswirkungen auf kindliche Entwicklung und kindliches Temperament. Die erste Studie (**Schrift I**) betrachtete lediglich die Pränatalphase, wobei es das Ziel war, Zusammenhänge zwischen mütterlicher subjektiver Stressbelastung und ihrem Kortisolniveau herauszufinden. Zusammenfassend liefern die Daten der ersten Studie Hinweise auf eine "vulnerable Phase" in der ersten Hälfte der Schwangerschaft, die eine Korrelation zwischen emotionalen und hormonellen Indikatoren der pränatalen Stressbelastung aufweist. Da Cortisol in dieser Studie nicht auf subjektive Belastungen der Mutter später in der Schwangerschaft reagieret, müssen andere Erklärungsansätze als die nach der „Fetal Programming Hypothese“ postulierte Aktivierung der HPA-Achse und die entsprechenden Ausschüttung von Cortisol in der Weiterleitung subjektiver Stressbelastung der Mutter auf den Fötus in der späten Schwangerschaft herangezogen werden. Es könnten andere Mediatoren zwischen mütterlicher subjektiver Reaktion und kindlicher Entwicklung, wie z. B. Endorphine, angenommen werden. Außerdem muss berücksichtigt werden, dass Stress auch durch nicht-hormonelle Wege übertragen und gepuffert werden kann, wie beispielsweise durch raschen Anstieg mütterlicher Herzfrequenz in Reaktion auf eine unbekannte Situation. Dies kann zu einer konditionierten Angstreaktion des Fötus führen. Insgesamt müssen komplexere Modelle als die „Fetal Programming Hypothese“ an-

genommen werden, die nicht-hormonelle und psychische Erklärungsansätze liefern, auf welchem Weg Stress der Mutter Einfluss auf die weitere kindliche Entwicklung nehmen kann. Diese Studie fügt die Erkenntnisse über die "sensibelste Phase" für das Verhältnis von emotionalem Stress und endokrinen Indikatoren für Stress während der Schwangerschaft hinzu. Zukünftige Studien sollten diese Tatsache berücksichtigen und bei der Untersuchung der Auswirkungen von pränatalem Stress auf die Nachkommen im ersten Trimenon der Schwangerschaft beginnen.

In der zweiten Studie (**Schrift II**) wurde die Frage beantwortet, ob mütterlicher Kortisolspiegel und kindliche Entwicklung mit fünf Monaten zusammenhängt. Die vorliegende Studie verdeutlichte eine differenzierte Analyse der Bedeutung der Zeit, wann Stress während der Schwangerschaft mit der Entwicklung des Kindes zusammenhängt. Dabei scheint das letzte Drittel der Schwangerschaft eine „sensible Phase“ in der Weiterleitung mütterlicher Stressbelastung auf kindliche Entwicklung zu sein. Die vorliegenden Ergebnisse sind im Einklang mit den Erkenntnissen von Huizink (Huizink, 2000) und Buitelaar (Buitelaar, et al., 2003). Dennoch sind diese Ergebnisse nicht mit der „Fetal Progamming Hypothese“ vereinbar, die annimmt, dass der Fötus in der zweiten Hälfte der Schwangerschaft vor mütterlicher Kortisolausschüttung über das Enzym 11ß- HSD II geschützt ist. Auch hier müssen komplexere Modelle zur Erklärung der Auswirkung mütterlicher Stressbelastung auf kindliche Outcome-Variablen postuliert werden.

In der letzten Studie (**Schrift III**) stellte sich heraus, dass Mütter, die am Ende der Schwangerschaft weniger gestresst waren, Kinder mit stärkerer Inhibition hatten. Erklärungen hierfür können nur spekuliert werden. Möglicherweise sind die Kinder von Müttern, die extrem wenig gestresst waren in der Schwangerschaft, nicht an neue Situationen und Reize und den dadurch entstehenden ‚Stress‘ gewohnt. Aus evolutionsbiologischer Sicht bedeutet dies möglicherweise eine pränatale Anpassung der Kinder an die Umgebung, die sie später postpartal vorfinden werden. Dies scheint einen evolutionären Vorteil für das Überleben der Spezies zu haben. Das bedeutet, dass Kinder, die in Zeiten hoher Belastung geboren sind, dazu neigen, ein Tempermanet zu zeigen, das weniger affektiv und weniger vorsichtig ist. Dem Sprichwort folgend: „Krieg bringt Krieger hervor“.

Insgesamt lässt sich in den Studien von Rothenberger et al. deutlich zeigen, dass Stress während der Schwangerschaft ein ernst zu nehmendes Thema ist. Es führt nicht nur zu körperlichen Veränderungen bei der Mutter, sondern kann die kindliche Entwicklung nachhaltig bis ins junge Erwachsenenalter beeinflussen. Pränatalstress wird auch für die Entwicklung von

psychischen Erkrankungen wie ADHS, sozialen Ängsten und Schizophrenien verantwortlich gemacht.

Es ist deshalb an der Zeit, Ärzte und Hebammen, die die medizinische Betreuung der schwangeren Frauen leisten, auf diese Zusammenhänge aufmerksam zu machen. Sie müssen die Frauen in der Schwangerschaft nicht nur für Ernährung, Sport und Gesundheitsverhalten wie Rauchen und Trinken sensibilisieren, sondern auch die Gesellschaft für die Belastung der Frau während der Schwangerschaft. Da Schwangerschaft an sich als Stressor wahrgenommen und häufig als Auslöser für verschiedene psychische Erkrankungen wie Depression und Psychosen verantwortlich gemacht wird, sind Schwangere per se besonders anfällig für Belastungen. Das Gesundheitssystem scheint noch weit davon entfernt zu sein, niederschwellige Angebote zur Prävention von Stress während der Schwangerschaft anzubieten. Dennoch scheint die Grundlage der Forschungsergebnisse darauf hinzudeuten, dass pränataler Stress Auswirkungen auf die kindliche Entwicklung der Nachkommen haben kann. Ungeachtet dessen sind wir in der Erforschung von Stressbelastungen während der Schwangerschaft, vor allem in Deutschland, noch ganz am Anfang. Weitere großangelegte prospektive Längsschnittstudien mit Risikogruppen, z. B. mit psychisch kranken Frauen, die schwangere Frauen mit ihren Kinder über mehrere Jahre begleiten, sind notwendig, um Zusammenhänge besser verstehen zu können und frühzeitige Interventionsstrategien für Mutter und Kind zu entwerfen.

Ein Folgeprojekt zur Nachuntersuchung der im Rahmen dieser Kohorte geborenen Kinder im Alter von drei Jahren ist geplant und wird, aufgrund der hohen wissenschaftlichen Bedeutung und der bisher hervorragenden Retentionsrate der Stichprobe, bei der Deutschen Forschungsgemeinschaft (DFG) beantragt.

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Anhang

Schrift I)

Running head: PRENTAL STRESS

Prenatal stress: Course and interrelation of emotional and physiological stress measures

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Keywords: Prenatal stress, Pregnancy, Perceived stress, pregnancy-related anxiety, marital satisfaction, critical life events, cortisol

Abstract

Background. Prenatal Stress is known to be a potential risk factor for cognitive, behavioural and motor development (Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2003; B. R. Van den Bergh & Marcoen, 2004) that even last until adolescence (B. R. Van den Bergh, Mennes, et al., 2005; B. R. van den Bergh, et al., 2006). A consensus of how 'Prenatal Stress' can be measured, in which trimester of pregnancy women should be studied and whether subjective feelings of being stressed are associated with a hormonal response is still lacking. To close this gap, a prospective longitudinal study was conducted in pregnant women. ***Sampling and Methods***. N= 108 subjects were asked to fill out questionnaires concerning pregnancy-related anxiety, perceived stress, marital satisfaction, critical life events and to collect salivary cortisol in each trimester of pregnancy. ***Results.*** Fear of giving birth increases until the end of pregnancy and marital satisfaction is highest at the end of pregnancy. Perceived stress is related to a hormonal response in cortisol only in the first ($r = .18$, $p < .10$) and second ($r = .18$, $p < .10$) trimester of pregnancy. Critical life events are linked to raised cortisol levels in early pregnancy only ($r = .28$, $p < .01$). ***Conclusion.*** Prenatal stress can be operationalised by using different subjective as well as physiological stress measures. Only in the first half of pregnancy self-report and physiological stress measures seem to be associated.

Introduction

Prenatal stress is a construct receiving growing scientific attention with regard to consequences for the offspring. Multiple effects have been reported: lower birth weight (Paarlberg, et al., 1995; Wadhwa, et al., 1993), prematurity (Dole, et al., 2003; Pagel, et al., 1990), smaller head circumference (Lou, et al., 1994; Lou, et al., 1992) and attentional disorders (Huizink, 2000; B. R. Van den Bergh, Mennes, et al., 2005) of the offspring. In a study of Diego and co-workers (Diego, et al., 2009) prenatal depression was shown to restrict fetal growth. A finding that is highly alarming in the light of the longitudinal adverse metabolic affects described for children who are small for gestational age. Also, it was shown by Moehler et al. (Moehler, et al., 2008) that maternal lifetime stress was associated with perinatal medical complications.

To date, only very few studies analyzed the factors with potential relevance as stressors for pregnant women. Pregnancy itself has been postulated to be a relevant stressful factor, specifically concomitant anxiety (Bjelica & Kapor-Stanulovic, 2004). Moreover, studies concerning prenatal stress and its outcome on the offspring focused on pregnancy-related anxiety (Huizink, 2000; Mancuso, Schetter, Rini, Roesch, & Hobel, 2004), depression (Bennett, Einarson, Taddio, Koren, & Einarson, 2004; Figueiredo & Costa, 2009), daily hassles (Buitelaar, et al., 2003), perceived stress (Davis, et al., 2007), support from the partner (Hohmann-Marriott, 2009; Lemola, Stadlmayr, & Grob, 2007) and physiological stress measures (de Weerth & Buitelaar, 2005), etc. Reck et al. (Reck, 2009) reported a significant relationship between anxiety and depression and discovered a prolonged delivery as a consequence of anxiety. Martini et al. (Martini, 2009) reported a high amount of up to 15% of clinically relevant depression in pregnancy, measured by the Edinburgh Postnatal Depression Scale (Cox, et al., 1987). Additionally, in a recent study - described by Figueiredo and colleagues (Figueiredo & Costa, 2009) – maternal depression predicted a worse emotional involvement before childbirth, while mother's anxiety predicted a worse emotional involvement with the infant after childbirth. Additionally, pregnant women with a worse emotional involvement with the offspring were at risk of poorer emotional involvement with the infant and higher anxiety and depression at three months postpartum.

Therefore, emotional stress in pregnancy is a factor of fundamental and longitudinal psychopathological significance. In line with the ‘Fetal Programming Hypothesis’ we assume that prenatal stress is programming the infant’s brain and its metabolism by the stress hor-

mone cortisol. According to this model, cortisol is the mediator of emotional stress and the alterations in the behavioural and emotional development of the child after stress exposure of the mother during pregnancy. However, studies regarding the association between self-reported and physiological stress measures are still rare, inconclusive and depend on the nature of stress, its timing during pregnancy and if basal or stress-induced hormones are examined (Glover, et al., 2009). Rieger (Rieger, 2005) found no association between subjective stress of the mother and salivary cortisol in the first trimester of pregnancy and negative association at the end of pregnancy. A recent study from Davis and Sandman (Davis & Sandman, 2010) reported no association between cortisol levels and pregnancy-related anxiety, depression and parenting stress in none of five study points during pregnancy. Wadhwa and colleagues found elevated salivary cortisol levels in stressed pregnant women compared to non-stressed pregnant women (Wadhwa, et al., 1996). Obel et al. detected that women in the last trimester of pregnancy who were more stressed had higher evening cortisol levels (Obel, et al., 2005). Compared to that, Huizink found association between morning cortisol levels and emotional stress in late pregnancy (Huizink, 2000). Zou and colleagues (Zou, et al., 2009) recently examined the correlation between psychological and hormonal parameters in the course of pregnancy. These authors described that the rates of anxiety and depression were higher in the first trimester and the postpartum groups. Depression was correlated with sharp changes in estradiol and progesterone levels and there was a correlation between depression and anxiety. Relevant changes in maternal hormone levels were correlated with depression; depression was correlated with anxiety; and anxiety was correlated with elevated cortisol levels. It has therefore been concluded that measuring maternal hormones may be a diagnostic tool to evaluate psychological stress and associated risks (Zou, et al., 2009).

In sum, we can state that maternal emotional stress in pregnancy is a factor of high relevance to child mental and physical development as well as mother-child-relationship and maternal postnatal psychopathology. Without corresponding data, one cannot conclude that cortisol levels might be an important mediator explaining how mothers' emotional experience affects the yet unborn child. The present report examines the course and interrelation of different self-report and physiological stress measures over the course of pregnancy. We hypothesize that pregnancy-related anxiety and more specifically fear of giving birth is highest at the end of pregnancy. Cortisol levels were assumed to increase over the course of pregnancy, being highest at the end of pregnancy. Associations between cortisol and subjective stress measures are presumed to be present.

Methods

Participants

The study was conducted between November 2007 and August 2009. The sample consists of healthy European pregnant women recruited in early pregnancy (week of gestation: 13.6±1.68) through local newspaper, homepages and in obstetricians' offices in Heidelberg and surrounding area. Exclusion criteria were (a) inability to speak and read German language, (b) twin pregnancy, (c) advanced pregnancy (>19 week of pregnancy), (d) inability to come to the laboratory at in infant's age of three and five months. The study protocol was approved by the ethic committee of the University Clinic of Heidelberg. All pregnant women were informed about the course and the aim of the study and gave written informed consent.

Out of 121 women contacted, 111 women were included in the first study wave during early pregnancy (gestational week 13.6±1.68). Ten women declined to take part at the study because of early abortion (3, 30%), lack of interest (6, 60%), or illness of the mother (1, 10%). Out of 111 women included in the first study wave, 104 women sent back their second data set in mid-pregnancy (gestational week 22.0± 2). Drop-out was because of abortion (1, 14.5%), loss of data during mailing (1, 14.5%), and forgetting to send back the data set in time (5, 71%). 106 women were included in the last study wave (gestational week 32.13±2) during pregnancy. Drop-out was because of handicap of the fetus (1, 33%) and forgetting to send back the data set in time (2, 67%). The final sample consists of 108 pregnant mothers between 17 and 42 years old when they get pregnant (M = 31.04, SD = 5.23). Seventy two per cent of our sample has "Abitur" (German university entrance qualification) or higher educational degree.

Study design

As part of a longitudinal study focusing on prenatal stress and its impact on child development, in each trimester of pregnancy the subjects were sent a variety of questionnaires concerning pregnancy-related anxiety, perceived stress, critical life events and marital satisfaction. Social support and prenatal emotional stress were assessed as well but are not part of this report. The women were asked to collect salivary cortisol in each trimester of pregnancy under controlled conditions.

Physiological stress measure.

Stress was detected by the physiological hormone response of the pregnant subjects in salivary cortisol. Basal salivary cortisol levels were collected using salivette collection devices (Sarstedt®, Germany). The subjects were asked to chew on cotton rolls for two to three minutes on three consecutive days between 11am and 1pm. Subjects were instructed to collect saliva out of their oral cavity in a quiet and non-stressed situation, store the salivettes in the refrigerator (-20°C) and send them back in a covered envelope after they collected three cortisol probes. The salivettes were centrifuged at 300rpm for 5 minutes and salivary-free cortisol concentrations were analyzed in the pharmacological laboratory of the University of Heidelberg (Germany).

Subjective stress measures.

The following questionnaires were used to measure 'subjective stress' of the pregnant mothers.

(a) The women were asked to fill out the *Pregnancy Anxiety Questionnaire-Revised* (PRAQ) (Huizink, 2000) that contains the subscales 'fear of giving birth', 'fear of having a handicapped child' and 'fear of one-self's unattractive appearance'. The PRAQ was developed by van den Bergh (B. Van den Bergh, 1990), revised by Huizink (Huizink, 2000) and translated in German language by Moehler et al. (Mohler, et al., 2006). Women answered on a four point Likert scale how often ("never", "seldom", "frequently", "mostly") they felt anxious about giving birth, having a handicapped child and their own unattractive appearance. Cronbach's alpha for all three subscale is >.76.

(b) The *Perceived Stress Questionnaire* (PSQ) from Levenstein et al. (Levenstein, et al., 1993) and translated by Fliege et al. (Fliege, Rose, Arck, Levenstein, & Klapp, 2001) contains four scales (worries, tension, joy, demands); differently from the original version by Levenstein et al. (Levenstein, et al., 1993) that includes five scales. The original 30 items were reduced to 20 items where the subjects are asked how often they feel calm, stressed, happy, worried, etc. Internal consistency of the subscales is ranging from .80 to .86; reliability is at least .80.

(c) The M*arital Satisfaction* was assessed using the German "Fragebogen zur Partnerschaftsdiagnostik" ('Questionnaire for diagnostics of partnership') (FPD) from Hahlweg (Hahlweg, 1996) with three subscales: behaviour during partnership conflicts, tenderness, and

commonness/ communication. Women had to indicate on a four point Likert scale how often ("never", "seldom", "often", "very often") some attitudes from the partner or themselves occur. For example "He blames me of failures I did in the past". Reliabilities for all subscales are located between .88 and .95. Internal Consistency for the whole scale constitutes r = .83.

(d) Women were asked in each trimester during pregnancy if they experienced *critical life events*, f. e. separation from partner, medical complication during pregnancy, financial problems, death of a relative, etc. All possible life events were summarized to one 'critical life event score' ranging from zero to eleven.

Statistical analysis

Kolmogorov-Smirnov-Test was investigated to test for normal distribution. The General Linear Model for repeated measures was assessed to test for changes over time in different questionnaires and in cortisol levels. Correlation between the different questionnaires and cortisol were conducted using Pearson or Spearman's rho correlation if data are non-normally distributed. A p-value of .05 was regarded as significant. Cohen's effect sizes (= d) were defined as the difference between a) T2 (second trimester) minus T1 (first trimester) (= d (T2-T1)) and b) T3 (third trimester) minus T2 (second trimester) (= d (T3-T2)) and divided trough the standard deviations of these differences respectively. Effect sizes were computed for each stress measure separately. The whole sample was split into three groups of 'low, medium and high stress' using the 25%-, 50%- and 75%- percentile of the cortisol level referring each woman to one special 'stress group'. We ran univariate ANOVAs examining differences between the three stress groups with respect to the different emotional stress measures for each time point separately. SPSS 17.0 version was used.

Results

Pattern of different stress measures over the course of pregnancy

The mean basal salivary *cortisol* levels (in ng/ ml) increase significantly over the whole course of pregnancy ($F(2, 98) = 26.94$, $p = .000$). There is a linear change over the course of pregnancy with highest cortisol levels at the end of pregnancy. Effect size for the difference between second and first trimester of pregnancy is d (T2-T1) = .55. Effect size for the time

comparison from last to second trimester is smaller (d (T3-T2) = .11). This increase of cortisol level is in accordance with theory as presented by several studies (Davis & Sandman, 2010; Obel, et al., 2005; Ruiz, Fullerton, Brown, & Schoolfield, 2001).

Pregnancy-related anxiety does not change significantly over the course of pregnancy (F (2, 100) = .33; p = .72). With regard to the subscales of the PRAQ (Huizink, 2000; Mohler, et al., 2006; B. Van den Bergh, 1990) only 'fear of giving birth' changes linearly over the course of pregnancy (F (1.82, 100) = 3.37, p = .029, Greenhouse-Geisser-correction) with highest fear of giving birth at the end of pregnancy. Effect sizes for time difference between T2- T1 and T3- T2 with regard to pregnancy- related anxiety are rather small (d (T2- T1) = -.03; d (T3-T2) = .09).

Perceived Stress measured by the PSQ from Levenstein et al. (Levenstein, et al., 1993) does not change significantly over the whole course of pregnancy (F (1.86, 100) = .96, p = .38, Greenhouse-Geisser-Correction). None of the subscales of the PSQ yielded significant results. Effect size for the time difference between T2 and T1 is negative (d (T2- T1) = -.16); for the time difference between T2 and T3 effect size is almost zero (d (T3-T2) = .09).

Marital satisfaction measured by the FPD from Hahlweg (Hahlweg, 1996) increases linearly (F (1.88, 94) = 12.18, p=.000, Greenhouse-Geisser-correction) over the course of pregnancy with highest marital satisfaction during the last trimester of pregnancy. The effect size for the time difference between T2 and T1 (d (T2-T1) = .33) is somewhat higher than the effect size for the difference until the end of pregnancy (d (T3-T2) = .11). With regards to the subscales of the FPD, the subscale 'partnership conflicts' differs significantly over the course of pregnancy (F (1.52, 97) = 34.23, p= .000, Greenhouse-Geisser-correction). There is a linear decrease of conflicts over the whole course of pregnancy with the lowest amount of conflicts in late pregnancy. 'Tenderness' also decreases linearly over the whole course of pregnancy (F (2, 95) = 6.6, p = .002) with highest scores of tenderness at the beginning of pregnancy. The subscale 'communication' does not change over the course of pregnancy (F (2, 96) = 2.26, p =.11).

Over the course of pregnancy there exists a marginally significant quadratic change of *critical life events* (F (2, 98) = 2.31, p = .10) with the lowest amount of critical life events in mid-pregnancy and most external stressors at the beginning of pregnancy. Effect sizes for the time difference between T1 and T2 (d (T2-T1) = -.19) and between T2 and T3 (d (T3-T2) = .11) are rather small.

All means and standard deviations of the different stress measures and the results of the General Linear Model for repeated measures are presented in table 1.

Table 1

Group comparison of different emotional stress measures in women with low, medium and high cortisol levels

The sample was divided in a 'low, medium and high stressed' group using the 25%-, 50%- and 75%- percentile of the cortisol level for each time point separately. In early pregnancy only, there exists a significant difference in the Perceived Stress Questionnaire (PSQ) with highest perceived stress levels in the group of women with extremely elevated cortisol levels ($F (2, 105) = 2.58$, $p = .03$). Furthermore, pregnancy-related anxiety was found to be highest in the group with highest cortisol levels in the first trimester of pregnancy ($F (2, 104) = 2.36$, $p = .10$) but not later on. All other time points and other emotional stress measures do not reach a level of significance. All means, standard deviations and results from the univariate ANOVA are reported in Table 2.

Table 2

Interrelation between different self-report stress measures and cortisol

Similarly to Huizink (Huizink, 2000; Mohler, et al., 2006) *Pregnancy-related anxiety* is not significantly associated with cortisol in none of the three trimester of pregnancy. With regard to the subscales of the PRAQ only worries about one self's unattractive appearance is marginally correlated with cortisol in mid-pregnancy ($r = .16$, $p =. 09$).

The association between *Perceived Stress* (Fliege, et al., 2001; Levenstein, et al., 1993) and cortisol has a tendency to be significantly associated in early (r = .18, p= .06) and second trimester of pregnancy (r = .18, p=.06). At the end of pregnancy this inter-correlation disappears (r = .05, p = .62). With regard to the subscales of the PSQ, the subscales 'worries' (r = .25, p = .01) and 'joy' (r = -.21, p =.03) are correlated with cortisol in the beginning of pregnancy. In mid-pregnancy, women who are more tense (r = .20, p= .04) and are more demanded (r = .22, p = .03) had higher cortisol levels. At the end of pregnancy no subscale is related to a hormonal response in cortisol.

Cortisol is not significantly associated with *marital satisfaction* over the whole course of pregnancy. The subscale 'conflict' has the highest but not significant interrelation to cortisol in the first trimester of pregnancy (r = .14, p = .16).

Only in the beginning of pregnancy *critical life events* are related with cortisol (r = .28, p = .000). More in detail, only separation from partner (r = .25, p = .01), financial problems (r = .25, p = .01) and 'other stress factors' (r = .24, p = .01) where subjects could insert their individual stress factors are correlated with cortisol in early pregnancy. As pregnancy advances there is no equivalent of critical life events in the hormonal response of the pregnant women. In Table 3 all interrelation between the different stress measures are presented.

Table 3

Discussion

The findings presented here show a differential pattern of stressors and a varying interrelation between emotional and hormonal indicators of stress over the course of pregnancy. Salivary cortisol, fear of giving birth and marital satisfaction increase over the whole course of pregnancy. The increase of salivary cortisol is in accordance with prior findings (Ruiz, et al., 2001). Obel and co-worker presumed that the increase of cortisol during pregnancy is related to a resetting of the sensitivity of the HPA axis in pregnant women (Obel, et al., 2005). Cortisol is elevated in early pregnancy because it might suppress the maternal immune system therewith the embryo will not be rejected from the maternal uterus (Pope, 1990). In late preg-

nancy cortisol among other hormones plays a crucial role for the fetal lung maturation and for preparation of the fetus for delivery (Hacking, et al., 2001; Ward, 1994).

Perceived stress and the amount of critical life events do not differ over the course of pregnancy which is in accordance with recent results presented by Davis and Sandman (Davis & Sandman, 2010). Moreover, fear of giving birth increases while pregnancy advances which is in accordance to findings reported by Huizink (Huizink, 2000). Davis and Sandman, in contrast, detected that pregnancy- related anxiety declines over the course of pregnancy (Davis & Sandman, 2010). Conflicts with the partner decrease over the course of pregnancy with highest amount of satisfaction with the partner at the end of pregnancy. These new results considering the partnership as one potential stress or buffering factor warrant further research. One may assume that this pattern of higher satisfaction and lower amount of conflicts may be helpful preparing the family to the arrival of a new family member.

Studies evaluating the association between endocrine and psychosocial indicators of maternal stress are highly inconclusive. In our study, we detected weak interrelation between emotional and hormonal stress measures only during the first and second trimester of pregnancy. Especially, the Perceived Stress Questionnaire (Levenstein, et al., 1993) and the amount of critical life events are reflected by hormonal oscillations during the first half of pregnancy. At the beginning of pregnancy, the amount of critical life events is highest, maybe as a result of highest medical complication in the first trimester of pregnancy. The Perceived Stress Questionnaire (PSQ) and the amount of critical life events seem to measure the result of a wide range of potentially stressful factors during pregnancy and may reflect a more direct picture of maternal emotional status than marital satisfaction. Therefore, association with cortisol might be the strongest for these measures in the first half of pregnancy. Marital satisfaction is not reflected by hormonal alterations pointing to the ‘indirectness’ of the measure: marital satisfaction is helpful and may buffer maternal stress via other than hormonal pathways. Marital satisfaction could also be counteracted by other factors, like fear of having a handicapped child or birth anxiety. Pregnancy-related anxiety and cortisol are not linked during pregnancy which is in accordance with prior studies (Huizink, 2000). Our results with weak or no association between raised maternal cortisol levels and psychological stress measures are compatible with findings from prior studies (Davis, et al., 2007; Davis & Sandman, 2010; de Weerth & Buitelaar, 2005; Petraglia, et al., 2001). According to the ‘Fetal Programming Hypothesis’, we could suggest that the child may be ‘buffered’ against maternal emotional stress in the second half of pregnancy. One potential reason for this buffering effect may be

the differential effects of the enzyme 11beta-Hydroxysteroid dehydrogenase type 2 (11ß-HSD 2) catalyzing the pathway from cortisol to cortisone, thereby deactivating its biological potential, showing an increase in activity during pregnancy. Therefore the biological consequences of stress in the mother (increase in cortisol) are not transmitted as directly to the fetus as they are in the first half of pregnancy. The protection of the 11ß-HSD2 barrier enzyme in the maternal placenta can also be destroyed by chronic stress in rats (Welberg, Thrivikraman, & Plotsky, 2005), which is highly alarming with regards to human studies. Another explanatory model suggests that increasing cortisol levels naturally occurring to constitute a ceiling effect with no potential for cortisol levels to further increase in response to stressors. As we do not include a high-risk group of pregnant women, we cannot verify this hypothesis with our data.

However, we have to keep in mind that our data and therefore our conclusion are limited for several reasons. First, we did not examine the entire spectrum of stress measures with regard to emotional (‘coping’, ‘personality’, ‘chronic stressor’, ‘daily hassles’ etc.) and physiological (dehydroepiandrosteron, estadriol, endorphins, oxytoxin, etc.) indicators of stress. Our data do not contain information about extreme emotional conditions: the sample studied here was a community sample of mentally healthy mothers. Therefore, it might well be postulated that women with mental illnesses or traumatic stress events in pregnancy do show a different pattern of hormonal-psychological interactions as cortisol levels might well be responsive to ‘extreme’ or ‘chronic’ emotional stress that was not described in the sample of this study.

Second, there exists no standardized procedure to measure salivary cortisol. There are varying approaches to measure salivary cortisol differing in time of collection saliva: some studies examine day curve cortisol (B. R. Van den Bergh, Van Calster, Smits, et al., 2008), morning and evening cortisol (Obel, et al., 2005) vs. cortisol collection on consecutive days as in our study. As we collected salivary cortisol between 11am and 1pm, we can not preclude that the link between emotional and physiological stress may only be visible in late pregnancy on evening measurements of cortisol. Evening cortisol is not as easily confounded by a ceiling effect as morning samples, as shown by Obel and colleagues (Obel, et al., 2005).

Thirdly, a variety of studies (Buitelaar, et al., 2003; O'Connor, et al., 2002; O'Connor, et al., 2003; Obel, et al., 2005) detected that cortisol in last trimester has crucial impact on the cognitive development of the child. Obel et al. (Obel, et al., 2005) found that evening cortisol values are negatively impacting the infant development. Huizink et al. (Huizink, et al., 2003)

reported that early morning cortisol levels in late pregnancy are important for this association. For this reason, we know that the last trimester of pregnancy plays a crucial role influencing the later development of the yet unborn child.

In summary, these data provide evidence for a ‘more vulnerable period’ in the first half of pregnancy with a link between emotional and hormonal indicators of stress in pregnant women. As cortisol levels do not respond to emotional information later in pregnancy in our data, other pathways than the HPA-axis and corresponding cortisol oscillations may be involved in forwarding emotional maternal stress to the fetus in late pregnancy. There might well be other transmitters of maternal emotional reactions, such as endorphins that was mentioned and assessed by Barbazanges et al. (Barbazanges, Piazza, Le Moal, & Maccari, 1996). Furthermore it should be taken into consideration that stress can be transmitted and buffered by non-hormonal pathways as well- a rapid increase in maternal heart rate in reaction to an unknown voice may just as well facilitate ‘conditioned’ anxiety reactions in the fetus. These questions are warranting future studies. However, this study adds the knowledge on the most ‘sensitive period’ for the relation of emotional stress and endocrine indicators of stress during pregnancy. Future studies should take this fact into consideration, and when studying the effects of prenatal stress on the offspring should start in the first trimester of pregnancy.

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Table 1. *Means, Standard deviations and significance of the GLM with repeated measures.*

	Mean	SD	Significance of GLM
Cortisol (in *ng/ ml*) T1	2.16	1.41	
Cortisol (in *ng/ ml*) T2	3.26	1.74	**
Cortisol (in *ng/ ml*) T3	3.45	1.68	
PRAQ T1	1.51	.46	
PRAQ T2	1.50	.39	n. s.
PRAQ T3	1.53	.42	
PRAQ: Subscale 'Fear of giving birth' T1	1.35	.54	
PRAQ: Subscale 'Fear of giving birth' T2	1.39	.44	*
PRAQ: Subscale 'Fear of giving birth' T3	1.48	.44	
PRAQ: Subscale 'Handicapped Child' T1	1.70	.70	
PRAQ: Subscale 'Handicapped Child' T2	1.61	.55	n. s.
PRAQ: Subscale 'Handicapped Child' T3	1.59	.63	
PRAQ: Subscale 'Appearance' T1	1.48	.68	
PRAQ: Subscale 'Appearance' T2	1.50	.66	n. s.
PRAQ: Subscale 'Appearance' T3	1.51	.65	
PSQ T1	39.75	20.52	
PSQ T2	37.87	20.54	n. s.
PSQ T3	39.03	21.66	
PSQ: Subscale 'Demands' T1	43.56	25.33	
PSQ: Subscale 'Demands' T2	41.32	26.47	n. s.
PSQ: Subscale 'Demands' T3	39.93	28.02	

PSQ: Subscale ‘Worries’ T1	28.05	23.89	
PSQ: Subscale ‘Worries’ T2	26.40	23.03	n. s.
PSQ: Subscale ‘Worries’ T3	29.77	25.27	
PSQ: Subscale ‘Joy’ T1	56.37	22.40	
PSQ: Subscale ‘Joy’ T2	57.10	21.78	n.s.
PSQ: Subscale ‘Joy’ T3	54.13	22.79	
PSQ: Subscale ‘Tension’ T1	43.76	23.82	
PSQ: Subscale ‘Tension’ T2	40.86	21.90	n. s.
PSQ: Subscale ‘Tension’ T3	40.53	23.26	
FPD T1	57.24	15.85	
FPD T2	62.01	17.71	**
FPD T3	63.02	16.97	
FPD: Subscale ‘Conflict’ T1	15.25	10.82	
FPD: Subscale ‘Conflict’ T2	9.27	9.83	**
FPD: Subscale ‘Conflict’ T3	7.20	8.54	
FPD: Subscale ‘Tenderness’ T1	20.57	6.28	
FPD: Subscale ‘Tenderness’ T2	19.76	7.04	**
FPD: Subscale ‘Tenderness’ T3	19.00	7.40	
FPD: Subscale ‘communication’ T1	21.88	5.27	
FPD: Subscale ‘communication’ T2	21.30	5.73	n. s.
FPD: Subscale ‘communication’ T3	21.22	6.10	
Critical life events T1	1.24	1.21	
Critical life events T2	1.00	1.17	n. s.
Critical life events T3	1.12	1.21	

Note: PSQ= Perceived stress questionnaire, PRAQ = Pregnancy-related questionnaire, FPD = Marital satisfaction questionnaire, Critical life events = amount of critical life events.

*p<.05, **p<.01, †p<.10, n. s. = non significant.

Table 2: *Different subjective stress measures in three groups of women with low, medium and high cortisol levels over the course of pregnancy.*

	PSQ			PRAQ			FPD			Critical life events		
Cort T1	MEAN	SD	Sign.	MEAN	SD	Sign.	MEAN	SD	Sign.	MEAN	SD	Sign.
1 = low	35.51	17.89		1.47	.38		58.83	16.17		.88	.95	
2 = med	40.22	20.81	*	1.48	.46	†	56.24	15.22	n.s.	1.23	1.15	n.s.
3 = high	50.45	23.36		1.71	.57		56.00	17.09		1.26	1.19	

	PSQ			PRAQ			FPD			Critical life events		
Cort T2	MEAN	SD	Sign	MEAN	SD	Sign	MEAN	SD	Sign	MEAN	SD	Sign
1 = low	32.80	20.78		1.42	.37		62.92	16.41		1.12	1.24	
2 = med	38.49	21.08	n.s.	1.56	.45	n.s.	59.02	20.53	n.s.	1.08	1.23	n.s.
3 = high	43.73	18.83		1.51	.32		61.28	18.18		.8	1.12	

	PSQ			PRAQ			FPD			Critical life events		
Cort T3	MEAN	SD	Sign	MEAN	SD	Sign	MEAN	SD	Sign	MEAN	SD	Sign
1 = low	38.47	24.75		1.52	.47		64.50	15.08		1.13	1.12	
2 = med	40.85	21.39	n.s.	1.61	.45	n.s.	63.63	15.87	n.s.	.90	.01	n.s.
3 = high	42.01	19.36		1.47	.32		58.95	19.67		1.38	1.35	

Note: Cort T1 = cortisol group divided by the 1= 25%-, 2= 50%-, 3= 75%- percentile during the first trimester of pregnancy, Cort T2 = cortisol group during the second trimester of pregnancy, Cort T3 = cortisol group during the third trimester of pregnancy.

SD = Standard deviation, Sign. = Significance of the unvariate ANOVA.

PSQ= Perceived stress questionnaire, PRAQ = Pregnancy-related questionnaire, FPD = Marital satisfaction questionnaire, Critical life events = amount of critical life events.

**p<.01, *p<.05, †p<.10, n. s. = non significant.

Table 3: *Inter-correlation between different questionnaires and cortisol over the course of pregnancy*.

	1 1st 2nd 3rd	2 1st 2nd 3rd	3 1st 2nd 3rd	4 1st 2nd 3rd	5 1st 2nd 3rd
1.PRAQ 1st 2nd 3rd	–				
2. PSQ 1st 2nd 3rd	 .34** .36** .26**	–			
3. FDP 1st 2nd 3rd	 -.08 n. s. -.32** -.08 n. s.	 -.28** -.33** -.41**	–		
4. Critical life events 1st 2nd 3rd	 .16 n. s. .29** .19†	 .40** .40** .27**	 -.04 n. s. -.08 n. s. -.19†	–	

5. Cortisol					–
1st	.13 n. s.	.18†	-.11 n. s.	.28**	
2nd	.14 n. s.	.18†	.00 n. s.	-.02 n. s.	
3rd	.01 n. s.	.05 n. s.	-.04 n. s.	.05 n. s.	

Note: 1st: First trimester, 2nd: Second Trimester, 3rd: Third Trimester of pregnancy.

PSQ= Perceived stress questionnaire, PRAQ = Pregnancy-related questionnaire, FPD = Marital satisfaction questionnaire, Critical life events = amount of critical life events.

**p<.01, *p<.05, †p<.10, n. s. = non significant.

Notification of Acceptance

Psychopathology
Ms. No. 200912019
Title: Prenatal stress: Course and interrelation of emotional and physiological stress measures

Dear Ms. Rothenberger,

We are pleased to inform you that the revised version of your manuscript has now been accepted for publication in Psychopathology and passed on to our production department from whom you will hear shortly.

We hope you will continue to submit work from your group to Psychopathology in the future.

Sincerely yours,

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Running Head: PRENATAL STRESS AND INFANT DEVELOPMENT

ORGINAL ARTICLE

Prenatal Stress and Human Infant Development

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Abstract

Background. There are several studies in rodents, primates but also in humans showing that prenatal stress has a potential impact on cognitive and emotional development in the offspring which may even last until adolescence (Huizink, et al., 2004; B. R. Van den Bergh & Marcoen, 2004; B. R. Van den Bergh, Mulder, Mennes, & Glover, 2005). Therefore this study investigated whether maternal stress in pregnancy is associated with impairment of infant cognitive or behavioural development. *Method.* N = 108 pregnant mothers collected salivary cortisol once during each trimester of pregnancy. N = 104 babies were tested with the Bayley Scales of Infant Development at an infant's age of five months. *Results.* Mothers' salivary cortisol levels at the end of pregnancy correlated negatively with mental developmental status of the offspring (r = -. 25, p = .01). Children of mothers in a high stress subgroup of pregnant women' perform worse in the Mental and Motor Scale of Bayley Scales of Infant Development (p <.05). *Conclusions.* Prenatal hormonal stress at the end of pregnancy seems to have a significant impact on the outcome of the offspring. These data strengthen the 'Fetal Programming Hypothesis' stating that maternal mental and hormonal state in pregnancy impacts early human development.

Key Practitioner Message:

- Prenatal Stress is a presumed risk factor for impaired cognitive and emotional development in the offspring
- The data strengthen the 'Fetal Programming Hypothesis' stating that maternal emotional stress stress during pregnancy is transmitted to the infant and thereby has an impact on the fetal brain, altering its normal development from early on.

Keywords: pregnancy, prenatal stress, cortisol, infant development, child development, Fetal Programming Hypothesis

Introduction

Evidence for an impact of prenatal stress on early behavioural and mental development in human infants has been recently provided (Buitelaar, et al., 2003; Huizink, et al., 2004; Huizink, et al., 2003). Psychological prenatal stress was found to be associated with changes in maternal cortisol levels and lower infant mental and motor scores as well as goal-directedness in later infancy (Mulder, et al., 2002). Psychological measures like the Pregnancy-related Anxiety Questionnaire in mid-pregnancy correlated with mental and motor development of the child at an infants' age of eight months. Furthermore, early morning value of cortisol at the end of pregnancy revealed an association with impairment of infants' developmental status at 3 and 8 months (Buitelaar, et al., 2003). The foci of primary interest in the studies looking at prenatal stress and outcome for the offspring are: infant temperament (de Weerth, et al., 2003; Huizink, et al., 2002), consequences affecting infant growth (Pagel, et al., 1990; Ruiz, et al., 2001), premature birth (Dole, et al., 2003; Wadhwa, et al., 1993), attentional organization (Huizink, 2000; Mohler, et al., 2006; B. R. Van den Bergh, Mennes, et al., 2005; B. R. Van den Bergh, Mulder, et al., 2005) and natural disaster (Yehuda, et al., 2008; Yehuda, et al., 2009). Correspondingly studies in primates and rodents have described differential impact of prenatal stress on the offspring (Barbazanges, et al., 1996; Kim, et al., 2001; Weinstock, 1997, 2005).

Efforts to elucidate the potential biological background of this phenomenon have primarily focused on rodents and primates: Infant behavioral alterations linked to maternal stress during pregnancy seem to be associated with changes in the hypothalamo-pituitary-adrenal response in rats (Henry, et al., 1994; Slamberova, Rimanoczy, Riley, & Vathy, 2004). Altered receptor densities in hippocampal sites have been proposed (Amiel-Tison, et al., 2004; McCormick, et al., 1995) as well as altered function of the amygdale (Becker, et al., 2007; Kim, et al., 2001).

Derived from these animal models the 'Fetal Programming Hypothesis' was confirmed assuming that the human fetal hypothalamic-pituitary-adrenal (HPA) axis is programmed during pregnancy. According to this hypothesis, prenatal events and stressors are programming the infant brain altering its normal neurodevelopment and behaviour (Kinsella & Monk, 2009). Studies looking at the fetal HPA and later development of the children of prenatally stressed mothers are still rare and have a variety of methodological weaknesses (Egliston, et al., 2007)

On a clinical level, a recent prospective longitudinal study (E. P. M. Brouwers, et al., 2001; Evelien P. M. Brouwers, Anneloes L. van Baar, & Victor J. M. Pop, 2001) on antenatal state/trait anxiety has provided evidence for an association between prenatal anxiety and externalizing problems in later childhood: high levels of antenatal anxiety in the second trimenon of pregnancy, explained 22% of the variance of ADHD–symptoms at age 8-9 years and 15 % of the variance of externalizing problems. In the AVON Longitudinal Study (B. R. Van den Bergh & Marcoen, 2004) prenatal anxiety assessed with a short screening questionnaire was found to double the risk for hyperactivity and inattention problems as well as conduct disorder at an age of 47 and 81 months. In the same study, maternal anxiety at 32 weeks of pregnancy was also found to be related to observer report measures of attention at 3 weeks and 12 months of age.

In the light of the actual relevance of these findings, specifically for the understanding of the pathogenesis of ADHD, this study examined in a sample of 104 human infants whether prenatal hormonal stress has impact on infant development.

Methods

Sampling

Data collection took part between November 2007 and August 2009. The sample will be described in more detail below. It consists of over hundred healthy European pregnant women recruited in early pregnancy (week of gestation: 13.6±1.68) through local newspaper, homepages and in obstetricians' offices in Heidelberg and surrounding area. Exclusion criteria were (a) inability to speak and read German language, (b) twin pregnancy, (c) advanced pregnancy (>19 week of pregnancy), (d) inability to come to the laboratory at an infant's age of three and five months. The study protocol was approved by the ethic committee of the university Clinic of Heidelberg. All pregnant women were informed about the course and the aim of the study and gave written informed consent.

Study design

A prospective longitudinal study was assessed collecting salivary cortisol in each trimester of pregnancy on three following days in a defined time interval under controlled conditions. At five month of age, infants' mental and motor development were tested by the Bay-

ley Scales of Infant Development (N. Bayley, 1993). Mothers brought their infants to the laboratory at a day-time when their infant was alert, fed and rested.

Measures

Prenatal stress measures. Stress was detected by the physiological hormonal response of the pregnant subjects in salivary cortisol. Basal salivary cortisol levels were collected using salivette collection devices (Sarstedt, Germany). The subjects were asked to suck on cotton rolls for two to three minutes on three following days between eleven and one o'clock in the morning. Subjects were instructed to collect saliva in a quiet, non-stressed situation before lunch time. They had to store the salivettes in the refrigerator (-20°C) and sent them back in a covered envelope after they collected three cortisol probes. The salivettes were centrifuged at 300rpm for 5 minutes and salivary-free cortisol concentrations were analyzed in the pharmacological laboratory of the University of Heidelberg.

Postnatal measures: Development of the child. Child Development was assessed via the Bayley Scales of Infant Development II according to Bayley (1993) at an infants' age of five months. The Bayley Scales of Infant Development include three subscales: Motor Scale, Mental Scale and Behaviour Rating Scale. The Mental and Motor Scales measure the infants' present stage of cognitive, language, personal-social, and fine and gross motor development. The Motor Scale measures the control of the gross and fine muscle groups and their functions: rolling, crawling and creeping, sitting, standing, walking, running and jumping; and supplementary fine motor handling involved in prehension, adaptive use of writing implements and the imitation of hand activities. The Mental and Motor Scale are nominal Dichotomy; The Behaviour Rating Scale is an ordinal scale and allows to assess the child's development on a 5-point- Likert scale, ranging from 1 (= worst coding) to 5 (= best coding). Two researchers trained for reliability independently observed the videotaped sessions for the Behaviour Rating Scale; Cohen's kappa was .88. Mental and Motor Scale were coded during the testing and was recoded by a second trained coder via the videotaped sessions. Cohen's kappa was .95.

Statistical analysis

Interrater-reliability for the development-ratings of the child was examined by intraclass correlation. Kolmogorov-Smirnov-Test was applied to test for normal distribution. Analyzing the alteration of prenatal cortisol over the course of pregnancy paired student's t-test was assessed. For each trimester of pregnancy correlation between prenatal stress and postnatal developmental status of the child was assessed by Pearson product-moment correlation for normally distributed data. Spearman's rho correlation was used for non-normally distributed data. Mann-Whitney U-test was assessed comparing group differences between women classified as 'low-stressed' and 'high-stressed' by median-split of the cortisol level in each trimester of pregnancy. Two sub- groups of 'extreme low-stress' and an 'extreme high-stress' were assessed by using the 25% and 75%-percentile of the cortisol level. Group differences for the infant development was tested by Mann Whitney U-Test. A p-value of .05 was regarded as significant. All calculations were done with the computer software SPSS 17.0.

Results

Sampling

Out of 121 women contacted, 111 women were included in the first study wave during early pregnancy (gestational week 13.6±1.68). Ten women declined to take part at the study because of early abortion (3, 30%), lack of interest (6, 60%), or illness of the mother (1, 10%). Out of 111 women included in the first study wave, N = 104 women sent back their second data set in mid-pregnancy (gestational week 22.0± 2). Drop-outs occurred due to abortion (1, 14.5%), loss of data during mailing (1, 14.5%), and forgetting to send back the data set in time (5, 71%). N = 106 women were included in the last study wave (gestational week 32.13±2) during pregnancy. Drop-outs in the last study wave can be explained because of handicap of the fetus (1, 33%) and forgetting to send back the data set in time (2, 67%). N = 104 mother-infant dyads came to the laboratory at an infant's age of five (M = 5.1 months, SD = .3) months. The final sample consists of 104 women ranging from 17 and 42 years of age (M = 31.04, SD = 5.23) at the beginning of their pregnancy. Seventy two per cent of our sample finished the highest normal school track in Germany and received their "Abitur" (equivalent to first year college).

Descriptive analyses of physiological prenatal stress measures

The mean basal salivary cortisol level (in ng/ ml) increases significantly over the course of pregnancy (t (102) = -6.39, p<.01). From early pregnancy (M = 2.12; SD = 1.37) to mid-pregnancy (M = 3.18; SD= 1.69) cortisol levels increase significantly (t (103) = -5.75, p<.01). From second to last trimester of pregnancy (M = 3.45; SD = 1.68) cortisol levels show further increase but this increase fails to reach level of significance (t (102) = -1.62, p=.11). The increase of cortisol over the course of pregnancy is in accordance with findings reported by Ruiz and colleagues (Ruiz, et al., 2001). It thus provides evidence suggesting that our measurement of physiological stress is valid.

Descriptive analyses of developmental status of the children

Mean mental developmental status of our sample is M = 95.57, SD = 9.12. Mean motor developmental status is M = 87.95, SD = 4.38. Mean Behavior Rating is M = 80.66, SD = 9.47. Comparing our results with norm data from Bayley (N. Bayley, 1993), we can conclude that our sample is somewhat but not significantly below the average mean mental and motor developmental status.

Correlation between prenatal stress and Infant development

Prenatal stress in the beginning of pregnancy is not correlated with Mental (r = -.001, p =.99), Motor (r = -.02, p = .86) or Behavior Rating Scale (r = .06, p = .53). Prenatal stress in mid-pregnancy is somewhat more associated with Mental (r = -.13, p = .24), Motor (r = -.12, p = .24) and Behavior Rating Scale (r = -.09, p = .41), but not significantly. Prenatal stress at the end of pregnancy is significantly correlated with Mental developmental status of the child (r = -.25, p = .01), but not significantly correlated with Motor Development (r = -.16, p = .13) and Behavior Rating Scale (r = -.06, p =.57).

Group comparison by median-split of the prenatal maternal cortisol level

We use the median-split of the cortisol level for each trimester of pregnancy to compare the group classified as ‘high-stressed’ and ‘low-stressed’ for the outcome of the developmental status of the children. Median cortisol for the first trimester of pregnancy is Median

= 1.76. In the group of ‘low-stressed’ women, mean cortisol level is M = 1.14, SD = .41, in the ‘high-stressed’ group Mean cortisol level is M = 3.14, SD = 1.31. We compare outcome of the child for these two groups. Motor developmental status of the ‘low-stressed’ group (N = 46) is marginally significant higher (M = 97.22, SD = 8.21) than in the ‘high-stressed’ group (N = 51; M = 93.73, SD = 9.82, U (94) = -.1.84, p = .07). Mental developmental status in the ‘low-stressed-group’ is also higher (M = 88.52, SD = 4.44) than in the ‘low-stressed’ group (M = 87. 27, SD = 4.36). But this difference does not reach a level of significance (U (94) = -1.48, = .14). For the Behavior Rating Scale, we can see the same pattern of results with higher mean values in the ‘low-stressed’ group (M = 80.85, SD= 10.41) in comparison to the ‘high-stressed’ group (M = 79.78, SD = 8.59), but not to a significant level (U (94) = -.99, p = .32).

In mid-pregnancy, median cortisol is 2.85 with mean cortisol level in the ‘low-stressed’ group of M = 1.85, SD = .59 (N = 47) and M = 4.55, SD = 1.29 in the ’high-stressed-group’ (N = 48). The Motor Scale is higher in the group of the ‘low-stressed’ group (M = 96. 09, SD =10.40) than in the ‚high-stressed‘ group (M = 94.98, SD= 8.10), but the difference does not reach a level of significance (U (94) = -1.16, p =.25). The difference in both groups looking at the Mental Scale does not reach a significant level (U (94) = -1.33, p =.18), so does the Behavior Rating Scale (U (94) = -.69, p = .49).

At the end of pregnancy, median cortisol level reaches a value of Median = 3.3. Cortisol level in the group of ‘low-stressed’ (N = 45) mothers is M = 2.21, SD = .69 and for ‘high-stressed’ (N = 49) mothers M = 4.77, SD = 1.37. Differences in both groups classified as ‘low- vs. high-stressed’ comparing Mental (U (93) = -1.49, p =.14), Motor (U (93) = -1.06, p = .29), and Behavior Rating Scales (U (93) = -.61, p = .54) does not reach a level of significance.

We can conclude that the only marginally significant differences between the ‘high- and low-stressed’ group exists when we compare the motor developmental status with prenatal cortisol in the first trimester of pregnancy. All other differences do not reach a level of significance. However, we can see that all differences tend in the expected direction. Mothers with high cortisol levels have children with retarded motor, mental and emotional development. Comparing the developmental status dividing the group via the median-split of the cortisol level does not give significant results. We assumed that medium stress levels do not have significant impact on infant development. For this reason, the next methodological step was to divide the group of subjects in a sub-group of ‘extremely low-stressed’ and ‘extremely high-

stressed' mothers by using the 25%- and 75% percentile of cortisol levels for each trimester of pregnancy. In this calculation, all subjects with medium cortisol levels will be excluded.

Extreme- group comparison

In first trimester of pregnancy, cortisol level of the 25%- percentile is 1.15, 50%-percentile = 1.76 and 75% = 2.83. We divided the group in an 'extreme high-stressed' group (N = 26) which mean cortisol level is M = 3.98, SD = 1.38 and an 'extreme low-stressed' group (N = 21) with a mean cortisol level of M = .79, SD= .28. The difference of these both extreme groups for prenatal stress at the beginning of pregnancy in the Mental (U (46) = -.833, p = .41), Motor (U (46) = -1.297, p = .19) and Behavior Rating Scale (U (46) = -.568, p = .57) does not reach a level of significance.

In mid-pregnancy, 25% percentile of cortisol is 1.85, 50% percentile is 2.85 and 75% percentile is 4.37. The group of 'extreme low-stressed mothers' (N = 24) has a mean cortisol level of Mean = 1.36, SD = .37, whereas the 'extreme high-stressed' group (N = 25) has a mean cortisol level of M = 5.58, SD = 1.09). In the Mental (U (48) = -1.30, p = .20), Motor (U (48) = -1.14, p = .25) and Behaviour Rating Scale (U (48) = -.78, p = .44), children of highly prenatal stressed mothers performing worse than children from prenatal non-stressed mothers. Anyhow, the group difference does not reach a level of significance.

At the end of pregnancy, 25% percentile of cortisol is 2.27, 50% percentile is 3.30 and 75% percentile is 4.47. The group of 'extreme low-stressed mothers' (N = 24) has a mean cortisol level of M = 1.70, SD = .55, whereas the 'extreme high-stressed' group (N = 25) has a mean cortisol level of M = 5.75, SD = 1.34. Children of mothers with a high cortisol level at the end of pregnancy perform significantly worse (M = 93.96, SD = 8.25) in the Motor Scale of Bayley Scales than children of mothers with a very low cortisol level (M = 97.63, SD = 12.04). This difference reach the level of significance (U (47) = -1.94, p =.05). Also, children from mothers with high cortisol level at the end of pregnancy perform significantly worse (M = 86.67, SD = 4.52) in the Mental Scale of Bayley Scale than children from mothers with very low cortisol levels (M = 89.67, SD =3.76, U (47) = -2.144, p = .032). In the Behavior Rating Scale, differences in the extreme groups does not reach a significant level (U (47) = -.88, p = .38), even though we can see that children of highly stressed mothers have lower Behaviour Rating scores (M = 80.29, SD = 8.26) than children from non-stressed mothers (M = 81.17,

SD = 10.26). All descriptive data of Mental, Motor and Behavior Rating Scale in the groups of ‘extreme highly stressed’ and ‘extreme non-stressed’ mothers over the course of pregnancy can be seen in Table 1-3.

Table 1

Table 2

Table 3

Discussion

In summary, we found that (1) prenatal maternal cortisol in the last trimester of pregnancy correlates negatively with Mental Development of the child, but not at an earlier stage of pregnancy. It seems that the influence of cortisol on the developmental status of the child increases over the course of pregnancy indicating that higher cortisol levels of the mother cause retarded motor and mental development of the child. (2) Group differences for median-split of the prenatal hormonal data do not reveal any significant differences concerning the outcome of the child. (3) Looking at extreme sub-groups using women who have highest and lowest cortisol levels (25%- 75%- percentile) during pregnancy, the comparison at the end of pregnancy but not earlier in pregnancy shows significant differences. According to our hypothesis, our data show that mothers with high prenatal cortisol levels have children who perform worse in the mental and motor development test at a postnatal age of five months than children from prenatal non-stressed mothers. These data are in line with the findings of the literature described above (Buitelaar, et al., 2003; Huizink, 2000; Mulder, et al., 2002; Wadhwa, 2005). However, what this study adds is a detailed knowledge on the time course of the association between maternal cortisol in pregnancy and infant development. As the studies cited above did not assess cortisol in each trimester of pregnancy or assessed cortisol only twice in pregnancy, they therefore cannot provide a differential analysis of the significance of time of stress onset in pregnancy. The latter seems to play a crucial role going in line with the findings from Huizink (Huizink, 2000) and Buitelaar (Buitelaar, et al., 2003). From these data we can conclude that prenatal hormonal stress of the mother at the end of pregnancy has a crucial impact on the mental and motor developmental status of the child at an infant's age of five months. It seems that looking at medium-stressed mothers no correlation between cortisol and child development can be found. This is predominantly present in a comparison of the extreme sub- groups.

These findings can be explained potentially by transmission of cortisol via the placenta and affecting infants' brain. This is supported in the literature by the following findings: Davis and coworkers (Davis, Townsend, et al., 2004) found that infants of mothers who had received prenatal cortisol treatment showed a blunted cortisol response to novel and stressful stimuli. Furthermore, it was shown that postnatal behavioural alterations induced by prenatal stress were depending on cortisol response and were not present to the same degree in the offspring of mothers who did not secrete cortisol in response to exogenous stressors because it was experimentally blocked (Barbazanges, et al., 1996).

Limitations of the validity of the present findings result from the fact that prenatal stress was only measured by salivary cortisol on three following days. We know that cortisol has a diurnal rhythm with highest levels in the morning and decreasing over the day. As we measured salivary cortisol between 11am and 1pm, we do not have a cortisol profile but only mean cortisol levels for each trimester of pregnancy. Furthermore, we only looked at prenatal cortisol responses of the mothers and its impact on child development. The correspondence of hormonal responses with subjective feelings of being stressed or whether emotional prenatal stress is influencing children's development is not looked at.

Furthermore, our data do not contain information about extreme emotional conditions: the sample studied here was a community sample of mentally healthy mothers. The effects of cortisol levels on children's developmental status may be even stronger when comparing clinical with community samples. In a clinical sample (f. e. migrants, people living in poverty, abused mothers); the range of hormonal and emotional stress may be higher than in the present sample. We can speculate that in these samples, prenatal stress might have even stronger effects on the development of the child and even from the beginning of pregnancy. For these reasons, our results can only be postulated for a 'normal sample' and might be even stronger in a clinical sample.

In the light of previous work and these data, further research on the impact of prenatal stress and human infant development seems to be of particular importance in order to identify specific targets for prevention of psychopathology, specifically ADHD.

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Table 1: Motor, Mental and Behavior Rating Scale of children five months of age of mothers classified prenatally as ‘extreme low-stressed’ and ‘extreme high-stressed’ in the beginning of pregnancy (T1).

T1	**Group**	**n**	**p**	**M**	**SD**	**df**
Motor Scale	Extreme low-stress	21	.19	97.52	7.05	46
	Extreme High-stress	26		94.73	8.63	
Mental Scale	Extreme Low-stress	21	.41	88.71	3.70	46
	Extreme High-stress	26		87.62	4.90	
Behaviour Rating Scale	Extreme Low-stress	26	.57	76.62	11.22	46
	Extreme High-stress	21		79.08	9.21	

T1: Group Classification by 25%- 75% percentile of prenatal cortisol level of the mothers at the beginning of pregnancy.

Motor Scale: Motor Scale of Bayley Scales of Infant Development, Mental Scale: Mental Scale of Bayley Scales of Infant Development, Behaviour Rating Scale: Behaviour Rating Scale of Bayley Scales of Infant Development.

Table 2: Motor, Mental and Behavior Rating Scale of children five months of age of mothers classified prenatally as ‘extreme low-stressed’ and ‘extreme high-stressed’ in mid- pregnancy (T2).

T2	**Group**	**N**	**p**	**M**	**SD**	**Df**
Motor Scale	Extreme low-stress	24	.25	96.17	12.18	48
	Extreme High-stress	25		94.96	7.18	
Mental Scale	Extreme Low-stress	24	.20	88.92	4.06	48
	Extreme High-stress	25		87.32	3.82	
Behaviour Rating Scale	Extreme Low-stress	24	.46	81.83	11.18	48
	Extreme High-stress	25		80.36	8.44	

T2: Group Classification by 25%- 75% percentile of prenatal cortisol level of the mothers in mid- pregnancy.

Motor Scale: Motor Scale of Bayley Scales of Infant Development, Mental Scale: Mental Scale of Bayley Scales of Infant Development, Behaviour Rating Scale: Behaviour Rating Scale of Bayley Scales of Infant Development.

Table 3: Motor, Mental and Behavior Rating Scale of children five months of age of mothers classified prenatally as ‘extreme low-stressed’ and ‘extreme high-stressed’ at the end of pregnancy (T3).

T3	**Group**	**N**	**p**	**M**	**SD**	**Df**
Motor Scale	Extreme low-stress	24	.05	97.63	12.04	47
	Extreme High-stress	24		93.96	8.25	
Mental Scale	Extreme Low-stress	24	.03	89.67	3.76	47
	Extreme High-stress	24		86.67	4.52	
Behaviour Rating Scale	Extreme Low-stress	24	.38	81.17	10.26	47
	Extreme High-stress	24		80.29	8.26	

T3: Group Classification by 25%- 75% percentile of prenatal cortisol level of the mothers at the end of pregnancy.

Motor Scale: Motor Scale of Bayley Scales of Infant Development, Mental Scale: Mental Scale of Bayley Scales of Infant Development, Behaviour Rating Scale: Behaviour Rating Scale of Bayley Scales of Infant Development.

Schrift III)

Running Head: PRENATAL STRESS AND INFANT AFFECTIVE REACTIVITY

Prenatal Stress and Infant Affective Reactivity at Five Months of Age

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Abstract

Background. Prospective studies concerning prenatal stress and its outcome on children's emotional development postulated potential influence of prenatal hormonal levels or emotional stressors on child development (Buitelaar, et al., 2003; Huizink, 2000; B. Van den Bergh, 1990). In a retrospective study, an influence of maternal emotional stress on infant affective reactivity was found (Mohler, et al., 2006).

Aims. This study was conducted in order to confirm these findings in a prospective study design.

Study design. A prospective longitudinal study design was conducted with three study waves during pregnancy and one time point five months postnatally.

Subjects. The final sample consisted of $n = 104$ mother- infant dyads.

Outcome measures. Maternal baseline cortisol levels and emotional stress were assessed in each trimester of pregnancy. Children were examined with the infant reactivity battery according to Kagan & Snidman (Kagan & Snidman, 1991) at the age of five months.

Results. Mothers of children with high affective reactivity (cry score ≥7) were significantly less depressed and perceived less stress ($p < .05$) in mid-pregnancy and perceived less stress ($p < .10$) and were confronted with less external stress factors ($p < .10$) at the end of pregnancy. Cortisol levels did not differ in both groups in any pregnancy trimenon ($p > .05$).

Conclusions. These data add a new specific aspect to the 'fetal programming hypothesis' and are the first to confirm the speculative data from retrospective studies. Baseline cortisol does not seem to be the 'hormonal mediator' of this association. Therefore, cortisol stress reactivity or other neuroendocrine mechanisms should be assessed in future studies.

Key words: Prenatal stress, Cortisol, Behavioural Inhibition, Infant reactivity

Introduction

Evidence for an impact of prenatal stress on early behavioural and mental development in human infants has been recently provided (Buitelaar, et al., 2003; Huizink, et al., 2004; Huizink, et al., 2003). The foci of primary interest in the studies looking at prenatal stress and outcome of the children are infant temperament (de Weerth, et al., 2003; Huizink, et al., 2002), consequences affecting infant growth (Ruiz, et al., 2001) and attentional organization (Huizink, 2000; Mohler, et al., 2006; B. R. Van den Bergh, Mennes, et al., 2005; B. R. Van den Bergh, Mulder, et al., 2005). Prenatal stress was widely studied in animal studies (Barbazanges, et al., 1996; Kim, et al., 2001; Weinstock, 1997, 2005). In human studies, indicators of basal prenatal maternal stress were measured by self-report (Dipietro, Costigan, et al., 2008; Huizink, et al., 2002; B. Van den Bergh, 1990) and physiological measures of prenatal stress, for example cortisol (Buitelaar, et al., 2003; Obel, et al., 2005; Wadhwa, et al., 1996) or other hormones (Zou, et al., 2009). One widely used approach to measure stress reactivity in humans is to examine natural disasters (Yehuda, et al., 2008; Yehuda, et al., 2009). Psychological prenatal stress was found to be associated with elevated maternal cortisol levels (Davis & Sandman, 2010; Obel, et al., 2005) and lower infant mental and motor score as well as goal-directedness in later infancy (Mulder, et al., 2002). Psychological measures like the Pregnancy-related Anxiety Questionnaire in mid-pregnancy correlated with mental and motor development of the child at an infants' age of eight months. Furthermore, early morning values of cortisol at the end of pregnancy revealed an association with impairment of infants' developmental status at 3 and 8 months (Buitelaar, et al., 2003). A recent study from Davis & Sandman (Davis & Sandman, 2010) detected that elevated cortisol levels in the beginning of pregnancy had deleterious consequences on children's development in the first year of life. However, elevated cortisol levels at the end of pregnancy were associated with accelerated cognitive development at 12 months of age.

Efforts to elucidate the potential biological background of this phenomenon have primarily focused on rodents and primates: Infant behavioural alterations linked to maternal stress during pregnancy seemed to be associated with changes in the hypothalamo-pituitary-adrenal response in rats (Henry, et al., 1994; Slamberova, et al., 2004). Altered receptor densities in hippocampal sites have been proposed (Amiel-Tison, et al., 2004; McCormick, et al., 1995) as well as altered function of the amygdala (Becker, et al., 2007; Kim, et al., 2001). Effects of stress on pregnant rats have also been examined by Braun and co-workers (Braun, 2006). The offspring of dams stressed prenatally developed fewer nerve connections in the cingulate cortex and the orbitofrontal cortex. In addition, the nerve cells in several other re-

gions showed different branching patterns to normal, with different effects on males and females. In the hippocampus, an important region that controls memory and emotion, males show an increase in branching while females show a decrease. In the prefrontal cortex, the males developed shorter nerve branches, while the females did not (Braun, 2006).

Studies linking childhood temperamental development with experiences of prenatal stress are rare but crucial and necessary. On a clinical level, a recent prospective longitudinal study (E. P. Brouwers, et al., 2001; E. P. M. Brouwers, et al., 2001) on antenatal state/trait anxiety has provided evidence for an association between prenatal anxiety and externalizing problems in later childhood: high levels of antenatal anxiety in the second trimester of pregnancy explained 22% of the variance of ADHD- symptoms at age 8-9 years and 15% of the variance of externalizing problems. In the AVON Longitudinal Study (B. R. Van den Bergh & Marcoen, 2004) - a prospective investigation study of 6493 subjects - prenatal anxiety, assessed with a short screening questionnaire, was found to double the risk for hyperactivity and inattention problems as well as conduct disorder at the age of 47 and 81 months. In the same study, maternal anxiety at 32 weeks of pregnancy was also found to be related to observer report measures of attention of infants at 3 weeks and 12 months of age (B. R. Van den Bergh & Marcoen, 2004).

Moehler et al. (Mohler, et al., 2006) conducted a study on infant reactivity assessed with the Kagan paradigm (Kagan, Reznick, & Snidman, 1988) and assessed prenatal stress by questionnaires retrospectively at two weeks postnatal age. In this study, they found that mothers with low levels of stress more often had infants with high affective reactivity, an infant's characteristic that has been linked to the fundamental temperamental trait of Behavioural Inhibition (Moehler, et al., 2008). Behavioural Inhibition has been defined as an inborn bias to respond to unfamiliar events with anxiety (Kagan, et al., 1989). A body of evidence has emerged stating that extremely inhibited toddlers are likely to exhibit inhibition with peers in later childhood (Hirshfeld-Becker, et al., 2007; Kagan, Resnick, & Snidman, 1987; Kagan, et al., 1989; Kochanska, et al., 1997) and even adolescence (Schwartz, et al., 1999). Also, inhibited children at 14 months have been shown to more often exhibit shy and withdrawn behaviour at age three and four and had a higher rate of social anxiety at age 7.5 and social phobia in adolescence (Biederman, et al., 1993; Biederman, et al., 1990; Hirshfeld, et al., 1992; Kagan & Snidman, 1991; Rosenbaum, et al., 1992). Fear or inhibition to unfamiliar events, people, and stimuli at 14 months can therefore be regarded as an important predictor for child and adolescent emotional development. Extreme inhibition at 14 months seems to put the child at risk for developing an internalizing disorder in later childhood (Rubin, Burgess, &

Hastings, 2002; Rubin, et al., 1997). Increased affective reactivity was shown to be related to the fundamental trait of behavioural inhibition (Moehler, et al., 2008) and might therefore play a role for longitudinal emotional development.

To date, knowledge about the origins of these early and long-lasting behavioural differences is limited. A moderate association with specific sites on the glutamic acid decarboxylase gene has been shown to be linked to some degree with behavioural inhibition in mice (Smoller, et al., 2001). Later, a modest relation between an allele of the CRH (corticotropin-releasing hormone) -linked locus and behavioural inhibition was observed by the same authors (Smoller, et al., 2003).

Moehler et al. (Mohler, et al., 2006) were the first to report an association of this fundamental temperamental trait with prenatal stress. Prenatal stress is highly relevant to the understanding of children's emotional development and the significance of prenatal stress. However, these findings described earlier were gathered in a retrospective study design i.e. baseline prenatal stress was assessed by questionnaires postnatally and cortisol levels during pregnancy were not tested. Because of the potential clinical relevance of the findings in this article the necessity was concluded to conduct a prospective study starting in the first trimester of pregnancy and assessing stress via basal hormonal and questionnaire measurements. Therefore this study aimed at confirming the association between prenatal stress and infant affective reactivity in a prospective design, starting in the first trimester of pregnancy.

Methods

Participants

Data collection took part between November 2007 and January 2010. The sample consisted of n = 104 healthy European pregnant women recruited in early pregnancy (week of gestation: M = 13.6, SD = 1.68) through local newspaper, home pages and in obstetricians' offices in Heidelberg and the surrounding area. Exclusion criteria were (a) inability to speak and read German language, (b) twin pregnancy, (c) advanced pregnancy (>19 week of pregnancy), (d) inability to come to the laboratory at an infant's age of three and five months. Infant reactivity was assessed at five months.

Out of 121 women contacted, 111 women were included in the first study wave during early pregnancy (gestational week 13.6±1.68). Ten women declined to take part at the study because of early abortion (3, 30%), lack of interest (6, 60%), or illness of the mother (1, 10%).

Out of 111 women included in the first study wave, *N* = 104 women sent back their second data set in mid-pregnancy (gestational week 22.0± 2). Drop-outs occurred due to abortion (1, 14.5%), loss of data during mailing (1, 14.5%), and forgetting to send back the data set in time (5, 71%). *N* = 106 women were included in the last study wave (gestational week 32.13±2) during pregnancy. Drop-outs in the last study wave can be explained because of handicap of the fetus (1, 33%) and forgetting to send back the data set in time (2, 67%). *N* = 104 mother-infant dyads came to the laboratory at an infant's age of five (*M* = 5.1 months, *SD* = .3) months. One infant was born preterm (gestational age: 34+4). Removing this participant whose child was born preterm did not alter any of the findings described in the result section. All other children (*N* = 103) were born at term. All demographic variables of the whole sample are presented in table 1. All pregnant women were informed about the course and the aim of the study and gave written informed consent.

Table 1

Study design

A prospective longitudinal study was conducted with three study waves during pregnancy, in each trimester of pregnancy and with two study waves at three and five months postnatally. Data from the second measurement point with five months postnatally were reported here. The pregnant subjects were instructed to collect baseline salivary cortisol in each trimester of pregnancy on three consecutive days between eleven a.m. and one p.m. under controlled conditions. A variety of questionnaires assessing prenatal stress of the mother in each trimester of pregnancy was used. Cortisol probes were collected at the same time when subjects filled out questionnaires. For each particular trimenon during pregnancy, salivettes and questionnaires were sent to the subjects. They had to fill out questionnaires and collect saliva at home and sent them back in an enclosed envelope. At five month of age, infants' affective reactivity was tested in the laboratory of the University of Heidelberg according to the reactivity battery according to Kagan and Snidman (Kagan & Snidman, 1991). Mothers brought their infants to the laboratory at a day-time when their infant was alert, fed and rested.

Measures

Prenatal physiological stress measures. Baseline salivary cortisol levels of the pregnant subjects were collected using salivette collection devices (Sarstedt®, Germany). The subjects were asked to suck on cotton rolls for two to three minutes on three consecutive days between eleven a.m. and one p.m. As –according to results published previously we wanted to measure general baseline stress and cortisol levels, we collected salivary cortisol on three consecutive days. Cortisol shows a diurnal rhythm that we could exclude by measuring basal cortisol levels in the same time period on three consecutive days. Collecting basal cortisol levels only once during each study point during pregnancy was also reported by a research group around Davis (Davis, et al., 2007). Subjects were instructed to collect saliva in a quiet, non-stressed situation before lunch time. They had to store the salivettes in the refrigerator (-12-18°C) and sent them back in a covered envelope after they collected three cortisol probes. According to Sarstedt® (Germany) cortisol is saveable in salivettes for more than one week without cooling. Therefore, defrosting of the salivette probes did not matter. Nevertheless, subjects were instructed not to mail the salivettes over weekend or public holidays. The salivettes were centrifuged at 300rpm for five minutes and salivary-free cortisol concentrations were analyzed in the pharmacological laboratory of the University of Heidelberg.

Prenatal psychosocial stress measures. The German version of the *Perceived Stress Questionnaire* (PSQ) from Levenstein et al. (Levenstein, et al., 1993) and translated by Fliege et al. (Fliege, et al., 2001) contained four scales (worries, tension, joy, demands); differently from the original version by Levenstein et al. (Levenstein, et al., 1993) it included only four scales. The original 30 items were reduced to 20 items where the subjects were asked how often they felt calm, stressed, happy, worried, etc. We were interested in studying the subjective experiences of d*epressive symptoms.* The internationally used and well-validated *Edinburgh Postnatal Depression Scale* (EPDS) (Cox, et al., 1987) screening on a four- point Likert scale for pre- and postnatal depressive symptoms was administered. Women indicated how they felt in the last seven days. We asked the subjects for 'overall objective stress' including a) *complication during pregn*ancy like medical complication during pregnancy, days of staying in hospital, b) '*critical life events'* (f. e. separation from the partner, death of a family member, loss of job, loss of accommodation, financial difficulties and other stressors like death of a pet, marriage, building a house, moving out and c) '*risk behaviour'* like drinking and smoking. All objective stress events were summed up to one 'objective stress score' ranging from 0- 16.

Postnatal temperament measure of the child. Infant's affective reactivity at five months of age was operationalized as crying/fretting reactions to novel stimuli according to Kagan and Snidman (Kagan & Snidman, 1991). Infants were seated in an infant chair in a supine (ap-

proximately 30°) position. After a one minute baseline period which served to confirm that the infant did not show any physical or emotional discomfort with being placed in the infant seat and did not show significant motor or crying/fretting activity, they underwent the battery according to Kagan and Snidman (Kagan & Snidman, 1991). This battery consists of novel visual, auditory and olfactory stimuli arranged to be increasing in their complexity or intensity. The whole procedure was videotaped. Two raters who had undergone intensive training, examined the videotapes for frequency of crying in the whole 20s interval following stimulus presentation. Before stimulus presentation the infants had to be in a motorically and emotionally quiet state in order to ascertain that the infant's reaction was caused by the stimulus and not by fatigue or boredom. Infants' affective reactivity was counted on a trial by trial basis. For each child an 'affective reactivity score' was built by summing up the number of stimulus presentations to which the infants reacted with crying or fretting that was coded with 1. As we had 16 stimulus presentations the 'affective reactivity score' was ranging between zero and 16. In the case of interruption because of excessive crying/ fretting or fussing the remaining trails were coded with 1. Motor reactivity was not coded in this report for several reasons. *First,* the results from the study from Moehler, Kagan, Oelkers-Ax al. (2008) indicate that affective reactivity i.e. crying when confronted with novel stimuli was associated with later childhood behavioral inhibition, but not motor reactivity. This finding was slightly deviant from previous reports describing behavioral inhibition to be predicted by crying AND motor reactivity. A trans-cultural effect for this difference has previously been discussed. Furthermore, in the study conducted by Moehler et al. (Mohler, et al., 2006) maternal prenatal emotional stress- assessed in retrospect- was significantly associated only with infant affective reactivity to novelty. Therefore the same measure was used as in the previous study, including only infant affective reactivity to novelty.

Infant coders were blinded to maternal prenatal stress scores as well as all other relevant covariates such as life events and depression. Interrater-reliability was estimated by recoding a subsample of infants by a second rater.

Statistical analysis

Interrater-reliability for the reactivity-ratings of the child was examined by intraclass correlation. Kolmogorov-Smirnov-Test was applied to test for normal distribution. Analyzing the changes of prenatal stress (cortisol, depression, perceived stress and critical life events) over the course of pregnancy paired Wilcoxon test was assessed. First, we tested for interrelation between prenatal indicators of stress (cortisol and self-report measures) and infant affective

reactivity by applying Product-Moment- or Spearman's Rho correlation if data were non-normally distributed. In a second step, the whole sample was divided by the infants' 'crying score' as the measure of infant affective reactivity. The group of children was divided by the arbitrary 6/7- cut-off with regard to their crying/ fussing behaviour in an approach derived from Kagan (Kagan, 1994). Another methodological approach was to compare extreme groups of affective reactivity. For this approach the sample was divided into three groups with regard to their affective reactivity (affective reactivity: 'low' = crying score = 0, 'medium': 1-6, 'low' ≥ 7). Both groups were compared according to their demographic variables via χ^2-test. According to their prenatal perceived stress level, prenatal baseline cortisol levels, antenatal depression score and the prenatal critical life event score both groups were compared via Mann-Whitney U-test and univariate ANOVA. A p-value of .05 was regarded as significant. All calculations were done with the computer software SPSS 17.0.

Results

Pattern of prenatal stress over the course of pregnancy

Cortisol. From early pregnancy to mid-pregnancy basal cortisol levels increased significantly (z (103) = -5.75, p <.01). From second to last trimester of pregnancy cortisol levels show further increase but this increase failed to reach a level of significance (z (102) = -1.62, p >.05). The increase of cortisol over the course of pregnancy is in accordance with findings reported by several studies (Davis & Sandman, 2010; Obel, et al., 2005; Ruiz, et al., 2001). It thus provides evidence suggesting that our measurement of basal cortisol seemed to be valid.

Edinburg Postnatal Depression Scale (EPDS, (Cox, et al., 1987)). During pregnancy *depressive symptoms* showed a slight decrease from first trimester over the second trimester and an increase until the third trimester of pregnancy. Whereas the first decrease was not significant (z (103) = .18, p >.05), the second increase was significant (z (103) = 2.86, p <.01).

Perceived Stress (PSQ). Overall *Perceived Stress* (Levenstein, et al., 1993) did not change significantly over the whole course of pregnancy (z (105) = .05, p >.05). Overall perceived stress decreased from first to the second trimester and increased again to the end of pregnancy. All differences did not reach a level of significance (p >.05). *External stressors.* The overall '*objective stress score'* decreased significantly from first to mid-pregnancy (z (103) = -1.83, p <.01) and raised up to the end of pregnancy, but this time not significantly (z (99) = -.93, p >.05).

All means and standard deviations of the prenatal stress parameters and results from the Wilcoxen test are presented in Table 2.

Table 2

Interrelation between prenatal stress parameters and infants' affective reactivity

We run bivariate correlation for each prenatal stress parameter and infants' affective reactivity score for each time point during pregnancy separately. Only prenatal perceived stress measured by the Perceived Stress Questionnaire from Levenstein (Levenstein, et al., 1993) yielded significant correlation with infants' affective reactivity in the second *(*r = -.31, p <.01) and third trimester *(*r = -.20, p <.05) but not in the first trimester of pregnancy (r = -.13, p >.05). Furthermore, in mid-pregnancy all subscales of the PSQ were significantly related with infants' affective reactivity in a negative way ('worries': r = -.21, p <.05; 'tension'*:* r = -.27, p <.01; 'demands'*:* r = -.35, p <.01). The subscale 'joy' was positively correlated with infants' affective reactivity (r = .23, p <.05). In the third trimester of pregnancy, only 'worries' (r = -.21, p <.05) and 'demands' (r = -.23, p <.05) correlated with infants' affective reactivity. All interrelation of the different stress parameters and infants' affective reactivity are reported in Table 3.

Table 3

Group differences in children with 'high and low affective reactivity'

First, we divided the group of children in two groups of children with high affective reactivity and low affective reactivity using the arbitrary 6/7 cut-off score and looked at group differences with regard to their prenatal stress parameters for each trimester separately. Mean infant affective reactivity ranged from zero to 16 with mean affective reactivity of M = 1.49 and SD = 2.83 in the whole sample. In the high affectively reactive group n = 7 (6.73%) children were included with a mean cry score of M = 10.0, SD = 1.63. N = 97 (93.27%) children belonged to the low affectively reactive group with a mean cry score of M = .86, SD = 1.64.

Group differences in children with 'high and low affective reactivity' in demographic variables. We compared both groups concerning their demographic variables by χ^2- test. Mothers who's babies were high affective reactive in the presence of a novel stimulus had lower educational level. The results of the comparison are presented in table 4.

Table 4

Group differences in children with 'high and low affective reactivity' concerning maternal prenatal stress

We will present the results for each trimester of pregnancy separately.

Differences at the beginning of pregnancy. In the first trimester of pregnancy, all group comparisons between mothers of children with high affective reactivity ('high criers') and children with low affective reactivity ('low criers') did not reach a level of significance even though trends can be detected with higher stress in mothers of children with low affective reactivity ('low criers').

Differences in mid- pregnancy. In mid- pregnancy, women with high affectively reactive children were significantly less depressed than mothers from low affectively reactive children (U (96) = -1.84, p <.10). Perceived stress in the second trimester of pregnancy differed significantly between the group of mothers with high affectively reactive infants comparing to mothers with low affective reactive children (U (96) = -2.20, p <.05). With regard to the subscale of the PSQ from Levenstein and co-workers (Levenstein, et al., 1993), we could detect that only the subscales 'worries' and 'demands' differed significantly (p <.05). The subscales 'tension' differed only marginally (U (96) = -1.92, p >.10) between both groups.

Differences at the end of pregnancy. Mothers of children with high affective reactivity had marginally significantly less perceived stress (U (100) = -1.80, p <.10) and less external stressors (U (99) = -1.8, p <.10).

For detailed descriptive data, please refer to Table 5.

Table 5

Extreme group comparison in children with 'high and no' affective reactivity with regard to maternal prenatal stressors.

In a second methodological approach we divided the group of children in three groups according to their affective reactivity to test for differences in both extreme groups of 'high affectively reactive' and 'low affectively reactive' children. Children with 'mid range affective reactivity' with a 'cry score' ranging between 2 and 6 were excluded from the further analysis. In the extreme group comparison, there were no significant group differences in *the beginning of pregnancy* concerning prenatal stress parameters comparing the group of high and low affectively reactive children. *In mid-pregnancy*, a trend appeared that showed that mothers of high affectively reactive children had less prenatal depressive symptoms (U (70) = -1.92, p <.10) and less prenatal perceived stress (U (70) = -2.60, p <.01). With regard to the subscales of the PSQ, only the subscale 'joy' yielded a marginally significant difference (U (70) = -1.76, p <.10). At *the end of pregnancy*, the same patterns occurred with lower amount of perceived stress (U (71) = -2.00, p <.05) and lower amount of external stress factors (U (71) = -1.82, p <.10) in mothers with high affectively reactive children. The subscales 'worries' and 'tension' yielded significant results (p <.05). All means, standard deviations and results from the Wilcoxon test and the univariate ANOVA are presented in Table 6.

Table 6

Discussion

In summary, we can conclude that in mid-pregnancy, prenatal perceived stress is negatively related to infant affective reactivity. Mothers of high affectively reactive children had less perceived stress (less worry, tension and demands but not more joy) and less depressive feelings during mid-pregnancy. At the end of pregnancy, women with high affectively reactive children had less external stressors and perceived less stress. In this analysis, the group with 'medium size affective reactivity' is excluded. When an ANOVA was examined and the middle group was also considered in the analyses there were somewhat different results. In the first trimester of pregnancy, there were no significant differences between the three groups as in the extreme group comparison of the paired sample t- test. In mid-pregnancy, the mar-

ginally different result disappeared. For perceived stress, we found significant differences in all subscales of the PSQ (joy, tension, demands, worries). At the end of pregnancy, only marginally significant difference appeared for the overall perceived stress score, as well for the subscale demands, worries and tension. The subscale joy was not different over the three groups in the ANOVA and the extreme group comparison. Differences in the analysis of the three groups via the omnibus test ANOVA and the extreme group comparison via the Mann Whitney U-test occurred because of the different degrees of freedom and the differences in comparing two or three groups at the same time.

These data were the first to confirm a study conducted by Moehler et al. (Mohler, et al., 2006) describing an influence of maternal emotional stress on infant temperament in a retrospective design in a true prospective design.

Davis and colleagues found correlations between baseline cortisol measured around two p.m. and infant negative reactivity measured by the IBQ (Gartstein & Rothbart, 2003) two months postpartum (Davis, et al., 2007). Other studies looked at pre- and postnatal predictors of infant temperament. Kaplan and co-workers (Kaplan, Evans, & Monk, 2008) found that maternal sensitivity, but not psychiatric status, predicted infant responsiveness. DeWeerth et al. reported that in the group of mothers with higher prenatal awakening cortisol levels at the end of pregnancy were children with more crying, fussing and negative facial expressions in an observation task in a small sample size (n = 17)(de Weerth, et al., 2003).

Contrary to other studies studying prenatal stress and outcome on child temperament in our study included a variety of prenatal stress measurements: self-report and physiological indicators of stress. Furthermore, we examined prenatal stress during three time points during pregnancy and tested for infant affective reactivity in a behaviour observing lab situation according to Kagan and Snidman (Kagan, Resnick, et al., 1987) that is shown to be more valid than parental reports of infant's temperament (Pauli-Pott, Mertesacker, & Beckmann, 2004). Our sample size was medium size n = 104. Only two participants dropped- out until the last study point. As we wanted to replicate the findings from Moehler et al. (Mohler, et al., 2006), we measured baseline salivary cortisol during a certain time period on three consecutive days. We did not apply a stress reactivity design as we wanted to test for prenatal baseline stress to be able to replicate these latter findings in a prospective study design.

What is the biological mechanism transmitting prenatal maternal stress to infant emotional development? Baseline cortisol was not associated with infant affective reactivity, however, this finding does not preclude the possibility that mothers of children with high affective reactivity do show altered neuroendocorine reactivity of stress hormones including

cortisol. Also, other hormonal pathways might be involved in the association reported here: researchers provide evidence that prenatal stress-induced alterations are caused by maternal endorphin release affecting the development of the fetus (Keshet & Weinstock, 1995). Furthermore, an increase in serotonin transporter expression in the brainstem after prenatal stress has been postulated (Slotkin, Barnes, McCook, & Seidler, 1996), a finding that is mentioned here because serotonergic activity in the brainstem is one of the hormonal factors considered to be involved in infant reactivity (Kagan, 1994). One of the most commonly replicated aspect is the finding that prenatal stress may lead to elevated levels of CRH-expression in the hippocampus and the amygdala, the latter being the locus that is supposed to be controlling infant reactivity to novel stimuli (Kagan, Reznick, et al., 1987).

From an evolutionary point of view a mechanisms adapting children prenatally to the conditions they are going to be born into might constitute an advantage to the species, i.e. in high stress times, children tend to have a temperament that is less affectively reactive and cautious. So that ‘war times might produce warriors’.

However, whether cortisol reactivity or other biological mediators are responsible for the association reported here needs to be elucidated in future studies, thereby revealing potentially important contributions towards the understanding of ‘fetal programming’ with regard to emotional development.

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Conflict of interest statement.

We disclose any financial and personal relationships with other people or organizations that could inappropriately influence or bias our work. We disclose any potential conflicts of interest; f. e. employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, grants and any other funding.

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Table 1: Demographic variables of the whole sample.

	Mean	*SD*
	n (%)	
N= 111 pregnant women		
Maternal age (in years)	31.04	5.23
Marital status (out of 111 women)		
Married	66 (59.5%)	
Single	39 (35.1%)	
Divorced/ separated	6 (5.4%)	
Birth order (out of 104 children)		
Primiparous	66 (63.47%)	
Multiparous	38 (36.53%)	
Maternal education (out of 111 women)		
‚Abitur' (first year college) or higher	80 (72%)	
Lower education level	31 (28%)	
N= 104 children		
Boys	61 (58.65%)	
Girls	43 (41.35%)	
Gestational age at birth (in weeks)	39.3	1.4
Birth weight (in *g*)	3381	490
Birth length (in *cm*)	51.65	2.37
Head circumference (in *cm*)	34.87	2.38
Delivery mode (out of 104 children)		
Spontanseously	67 (64.42%)	
Cesarean section	28 (26.92%)	
Vacuum extraction	9 (8.66%)	

Table 2. Descriptive data of all prenatal stress measures with results from paired sample t-test (Wilcoxon).

	Mean	*SD*	*z*	*p*
Cortisol T1	2.12	1.37		
Cortisol T2	3.18	1.69	5.75	**
Cortisol T3	3.45	1.68	1.62	n. s.
EPDS T1	7.71	5.44		
EPDS T2	7.16	5.45	.18	n. s
EPDS T3	8.64	6.04	1.86	**
PSQ T1	41.44	21.25		
PSQ T2	38.22	20.48	.97	n. s.
PSQ T3	40.20	21.86	-.50	n. s.
Demands T1	44.81	25.22		
Demands T2	41.54	26.11	.88	n. s.
Demands T3	42.00	27.78	-.46	n. s.
Worries T1	30.33	25.42		
Worries T2	27.05	23.24	1.05	n. s.
Worries T3	31.19	24.68	-2.27	*
Joy T1	55.02	23.43		
Joy T2	56.79	21.90	-.33	n. s.
Joy T3	53.02	22.94	1.74	†
Tension T1	45.65	23.88		
Tension T2	41.09	21.71	1.80	†
Tension T3	41.64	23.51	-.27	n. s.
External stressors T1	1.28	1.37		
External stressors T2	3.18	1.69	-5.75	**
External stressors T3	3.45	1.68	1.62	n. s.

Note: *p* = level of significance: n. s. = non significant, †*p*<.10, **p* <.05, ***p* <.01.

T1 = first trimester, T2 = second trimester, T3 = third trimester of pregnancy.

z = paired sample Wilcoxon test.

EPDS = Edinburgh Postnatal Depression Questionnaire.

PSQ = Perceived stress questionnaire, demands, worries, joy, tension are subscales of the PSQ.

Table 3: Inter-correlation between different prenatal stress parameters and infant's affective reactivity.

	1 1st 2nd 3rd	2 1st 2nd 3rd	3 1st 2nd 3rd	4 1st 2nd 3rd	5 1st 2nd 3rd
1. EPDS 1st 2nd 3rd	–				
2. Cort 1st 2nd 3rd	.21** .04 n. s. .07 n. s.	–			
3.PSQ 1st 2nd 3rd	.68 **. .64** .78**.	.18† .18† .05 n. s.	–		
4. External stressors 1st 2nd 3rd	.33** .37** .23*	.28** -.02 n. s. .05 n. s.	.40** .40** .27**	–	

5. Affective Reactivity					–
1st	-.008 n. s.	-.05 n. s	-.13 n. s.	-.12 n. s.	
2nd	-.12 n. s.	.02 n. s.	-. 31**	-.09 n. s.	
3rd	-.06 n. s.	.09 n. s.	-.20*	-.10 n. s.	

Note: p = level of significance: n. s. = non significant, †*p*<.10, **p* <.05, ***p* <.01.

1st: first trimester, 2nd: second trimester, 3rd: third trimester of pregnancy.

PSQ= Perceived stress questionnaire, Cort = Cortisol, EPDS = Edinburgh Postnatal Depression Scale, Affective Reactivity = Infant's affective reactivity at five months posntatally.

Table 4: Differences in demographic variables in the groups of ‘high and low affective reactive’ children.

			χ^2
Sex	Girls	Boys	
High AR	4	3	
Low AR	56	40	.99
Birth order	Primiparous	Multiparous	
High AR	6	1	
Low AR	59	37	.26
Maternal education	Higher education	Lower education	
High AR	3	4	
Low AR	73	23	.04
Maternal age	< 25	> 25	
High AR	11	85	
Low AR	1	6	.71

Table 5: Group comparison in the group of 'high and low' affectively reactive children concerning prenatal stress parameters (cut-off score ≥ 7).

	Mean	*SD*	*U*	*P*
Infant's Affective Reactivity (AR)				
high (*n* = 7)	10.0	1.63		
low (*n*= 97)	.86	1.64	-5.18	**
Cort T1				
high AR (*n* = 6)	2.55	1.41		
low AR (*n* = 91)	2.18	1.41	-.41	n. s.
EPDS T1				
high AR (*n* = 7)	4.86	5.24		
low AR (*n* = 95)	7.87	5.43	-1.36	n. s.
PSQ T1				
high AR (*n* = 7)	30.72	24.01		
low AR (*n* = 96)	41.88	20.70	-1.31	n. s.
External stressors T1				
high AR (*n* = 7)	1.13	1.02		
low AR (*n* = 95)	1.29	1.23	-20	n. s.
Cort T2				
high AR (*n* = 7)	2.99	1.19		
low AR (*n* = 88)	3.24	1.77	-.18	n. s.

EPDS T2				
high AR (*n* = 7)	3.71	4.35		
low AR (*n* = 90)	7.31	5.49	-1.84	†
PSQ T2				
high AR (*n* = 7)	20.95	15.21		
low AR (*n* = 90)	29.31	20.57	-2.20	*
Subscale Demands T2				
high AR (*n* = 7)	18.10	16.20		
low AR (*n* = 90)	43.31	26.54	-2.46	*
Subscale Worries T2				
high AR (*n* = 7)	10.47	13.80		
low AR (*n* = 90)	27.85	23.45	-2.2	*
Subscale Tension T2				
high AR (*n* = 7)	24.76	21.68		
low AR (*n* = 90)	42.30	21.88	-1.9	*
Subscale Joy T2				
high AR (*n* = 7)	69.52	21.03		
low AR (*n* = 90)	56.30	21.78	-1.4	n. s.
External stressors T2				
high AR (*n* = 7)	.57	.79		
low AR (*n* = 90)	1.04	1.20	.89	n. s.
Cort T3				
high AR (*n* = 6)	3.61	2.04		

low AR (*n* = 89)	3.53	1.67	-.38	n. s.
EPDS T3				
high AR (*n* = 6)	6.17	7.11		
low AR (*n* = 95)	8.84	6.11	-1.12	n. s.
PSQ T3				
high AR (*n* = 6)	25.00	21.00		
low AR (*n* = 95)	41.30	21.89	-1.80	†
Subscale Demands T3				
high AR (*n* = 6)	27.79	30.82		
low AR (*n* = 95)	42.12	27.61	-1.34	n. s.
Subscale Worries T3				
high AR (*n* = 6)	15.56	15.00		
low AR (*n* = 95)	32.28	25.29	-1.69	†
Subscale Tension T3				
high AR (*n* = 6)	24.44	22.97		
low AR (*n* = 95)	42.81	23.69	-1.83	†
Subscale joy T3				
high AR (*n* = 6)	67.78	24.47		
low AR (*n* = 95)	52.00	24.37	-1.48	n. s.
External stressors T3				
high AR (*n* = 6)	.33	.52		
low AR (*n* = 94)	1.15	1.12	-1.83	†

Note: p = level of significance: n. s. = non significant, †*p*<.10, **p* <.05, ***p* <.01.

T1 = first trimester, T2 = second trimester, T3 = third trimester of pregnancy.

U = Mann-Whitney- U-Test for group comparison.

AR = Infant's Affective Reactivity at an infant age of 5 months.

Low affective reactivity: cry score = 0-6, high affective reactivity: cry score ≥ 7.

EPDS = Edinburgh Postnatal Depression Questionnaire. PSQ = Perceived stress questionnaire, demands, worries, joy, tension are subscales of the PSQ.

Table 6. Extreme Group comparison in the group of 'high, medium and low' affectively reactive children concerning prenatal stress parameters (cut-off ≥7).

	Mean	*SD*	*F U*	*P*
Affective Reactivity				
low AR (*n* = 67)	0.00	0.00		
medium AR (*n* = 30)	2.87	1.76	341.18	**
high AR (*n* = 7)	10.00	1.63	-8.53	**
Cort T1				
low AR (*n* = 64)	2.29	1.56		
medium AR (*n* = 29)	1.90	.90	.98	n. s.
high AR (*n* = 6)	2.56	1.68	-.28	n. s.
EPDS T1				
low AR (*n* = 67)	7.69	5.27		
medium AR (*n* =29)	5.77	5.77	1.71	n. s.
high AR (*n* = 7)	5.24	5.24	-1.25	n. s.
PSQ T1				
low AR (*n* = 67)	43.06	20.17		
medium AR (*n* = 30)	39.33	21.64	1.27	n. s.
high AR (*n* = 30)	30.71	16.97	-1.41	n. s.
External stressors T1				
low AR (*n* = 66)	1.44	1.28		
medium AR (*n* = 30)	1.03	1.10	1.21	n. s.
high AR (*n* = 7)	1.14	1.07	-.50	n. s.
Cort T2				
low AR (*n* = 63)	3.25	1.82		
medium AR (*n* = 27)	3.22	1.61	.07	n. s.
high AR (*n* = 7)	2.99	1.19	-.16	n. s.
EPDS T2				
low AR (*n* = 64)	7.59	5.58		
medium AR (*n* = 26)	6.70	5.21	-1.84	n. s.
high AR (*n* = 7)	3.71	4.35	-1.92	†

PSQ T2				
low AR (*n* = 64)	42.81	19.85		
medium AR (*n* = 27)	30.74	19.73	6.56	**
high AR (*n* = 7)	20.93	15.21	-2.60	**
Subscale Demands T2				
low AR (*n* = 64)	48.33	26.15		
medium AR (*n* = 27)	31.11	23.38	7.85	**
high AR (*n* = 7)	18.10	16.19	-2.66	**
Subscale Worries T2				
low AR (*n* = 64)	30.31	23.33		
medium AR (n = 27)	22.22	22.72	3.12	*
high AR (*n* = 7)	10.48	13.80	-2.49	*
Subscale Tension T2				
low AR (*n* = 64)	45.72	22.06		
medium AR (*n* = 27)	33.83	18.94	7.85	**
high AR (*n* = 7)	24.76	21.68	-2.20	*
Subscale Joy T2				
low AR (*n* = 64)	53.13	20.37		
medium AR (*n* = 27)	64.20	23.10	3.84	*
high AR (*n* = 7)	69.35	21.03	-1.76	†
External stressors T2				
low AR (*n* = 66)	1.09	1.19		
medium AR (*n* = 29)	.96	1.22	.66	n. s.
high AR (*n* = 6)	.57	.79	-1.03	n. s.
Cort T3				
low AR (*n* = 63)	3.41	1.73		
medium AR (*n* = 29)	3.83	1.49	.59	n. s.
high AR (*n* = 6)	3.61	2.04	-1.16	n. s
EPDS T3				

low AR (*n* = 66)	8.8	5.88		
medium AR (*n* = 29)	8.69	6.71	.59	n. s.
high AR (*n* = 6)	6.17	7.11	-1.12	n. s.
PSQ T3				
low AR (*n* = 66)	43.46	21.17		
medium AR (*n* = 29)	36.38	23.06	2.66	†
high AR (*n* = 6)	25.00	21.00	-2.00	*
Subscale Demands T3				
low AR (*n* = 66)	45.45	26.15		
medium AR (*n* = 29)	34.48	23.38	2.37	†
high AR (*n* = 6)	27.78	16.19	-1.63	n. s.
Subscale Worries T3				
low AR (*n* = 66)	35.05	25.50		
medium AR (*n* = 29)	25.98	24.06	2.66	†
high AR (*n* = 6)	15.56	15.00	-1.97	*
Subscale Tension T3				
low AR (*n* = 66)	44.75	22.06		
medium AR (*n* = 29)	38.39	18.94	2.44	†
high AR (*n* = 6)	24.44	21.68	-1.97	*
Subscale Joy T3				
low AR (*n* = 66)	51.41	21.04		
medium AR (*n* = 29)	53.33	26.49	1.41	n. s.
high AR (*n* = 6)	67.78	24.99	.13	n. s.
External stressors T3				
low AR (*n* = 66)	1.20	1.18		
medium AR (*n* = 29)	1.10	1.01	1.67	n. s.
high AR (*n* = 6)	.33	.52	-1.82	†

Note: *p* = level of significance: n. s. = non significant, †*p*<.10, **p* <.05, ***p* <.01.

T1 = first trimester, T2 = second trimester, T3 = third trimester of pregnancy.

F = univariate ANOVA over all affective reactivity groups. *U* = Mann-Whitney- U-Test for extreme group comparison.

AR: Infant's affective reactivity at an infant's age of five months.

Low affective reactivity: cry score = 0, medium affective reactivity: cry score = 2- 6, high affective reactivity: cry score ≥ 7.

EPDS = Edinburgh Postnatal Depression Questionnaire.

PSQ = Perceived stress questionnaire, demands, worries, joy, tension are subscales of the PSQ.