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DIPLOMARBEIT

Titel der Diplomarbeit

Effects of gestational stress on maternal performance and offspring growth and behaviour in the guinea pig (*Cavia aperea f. porcellus*)

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Abstract

Stress during pregnancy persistently alters ("programs") the offspring's phenotypic outcome. An adaptive significance, serving to maximise survival and reproductive fitness in a poor quality-environment experienced by the mother, has been proposed. Despite being a well documented phenomenon, little is known about the pathways of modulation. While stress-induced alterations in the maternal endocrine milieu have been commonly suspected to act as environmental cue to the fetus, more recent research suggest an involvement of postnatal factors as well. Hence, unfavourable environmental conditions are presumed to cause a shift in maternal investment; moreover, variations in care-giving activities of the mother affect not only physical maturation but have organising effects for example on brain differentiation processes in the neonate.

This study aimed to examine the consequences of challenging experiences during gestation for maternal performance in terms of behavioural mother-pup interactions and lactational effort in the domestic guinea pig (*Cavia aperea f. porcellus*), and its importance for phenotypic plasticity. Six adults were submitted to stress procedures, applied on a weekly basis covering mating and the early gestational period, while another group was left to breed undisturbed. In the offspring, growth rates over the lactation period and fear reactivity, as one of the parameters of the brain functional development, were monitored. Gestationally stressed dams engaged tendencially less time in pup contact and nursing, which was accompanied by significantly enhanced rejection behaviour and lowered milk yield. This may represents a strategy to advance infant weaning following demanding conditions in pregnancy. In the progeny of gestationally stressed mothers, slightly altered growth rates in females and tendencies towards heightened fear behaviour were evident. Even though the relevance of pre- and postnatal factors remains elusive and the extreme precociality of guinea pig young may reduce infant sensitivity to early care-taking, this work supports the hypothesis that altered maternal effort may be an important factor for the determination of stress-driven developmental traits.

Keywords: prenatal stress / Guinea pigs / maternal care / lactation / growth / stress response

Introduction

Exposure to stressful or fear-evoking conditions during gestation exerts complex influences on the offspring's phenotypic outcome, demonstrated in a wide range of animal species. This process is referred to as early life "programming" and involves the activity of non-genetic maternal factors on organisational pathways of differentiation processes in various targets, such as for example the central nervous system (Levine, 1994; Barker, 1999). Programming effects range from altered behaviour and endocrine functions to physiological development. For instance, delayed puberty (Harvey and Chevins, 1987), suppressed exploratory and locomotor activity (Barlow et al., 1987) and altered metabolic regulation (Vallee et al., 1996) have been previously assessed in the offspring of laboratory rodents subjected to chronic or short-term stress during pregnancy.

Several authors propose an adaptive significance of early life programming (e.g. Meaney, 2001; Kaiser and Sachser, 2005). Adaptations are not restricted to the process of natural selection but include inter-individual differences in phenotypes as a function of the prevailing environment (Agrawal, 2001; Cameron et al., 2005). The potential for plasticity of physiological systems allows environmental signals to set developmental trajectories that adjust the organism to an anticipated level of demand in later life (Dudley and Smitt, 1996). From an evolutionary perspective, it is conclusive that maternal stress determines the expression of phenotypic traits and life-history decisions which serve to maximise survival and reproductive success in an adverse environment of poor quality experienced by the mother (Meaney, 2001). Under conceptualisation of environmental perturbations such as high risk of predation, behavioural adjustments of exploration activity, for instance, are supposed to enhance offspring fitness (Caldji et al., 2000; Cameron et al., 2005).

A critical question in the context of environmental programming concerns the pathways mediating the influence of stressful events experienced by the mother on offspring development. Stress-induced changes in the maternal endocrine milieu have been theorised to directly affect fetal development as glucocorticoids, secreted by the mother, are known to pass the placental barrier (Zarrow et al., 1970). On the one hand, glucocorticoids are indispensible for normal maturation processes (Liggins, 2000); however, excess fetal exposure during sensitive windows of ontogenetic development exerts wide-ranging effects with permanent alterations in neuroendocrine function and behaviour (Uno et al., 1990). Maternal influence extends after birth and postnatal factors have to be taken into account as well (Power and Moore, 1986; Peters, 1988). In the mammalian young, the predominant component during early postpartum life constitutes maternal care, which is of central importance not only for nurture and physical maturation of the neonate but also for social and emotional development (Fleming et al., 1999; Wewers et al., 2003). Milk provision by the mother, as nearly the sole source of nutrition during infancy, has strong repercussions on life trajectories such as growth and presumably fertility as age at first reproduction correlates with body mass (Mellish et al., 1999). Most extensively studied in the rat, the tactile stimulation for the young regulates pup physiology and is crucial for neurobiological development, with influences persisting into adulthood (Fish et al., 2004). Experimental evidence for the role of maternal care in offspring maturation processes is commonly derived from deprivation and handling studies. Daily periods of extended separation from the mother induces cognitive and behavioural changes with frequently reported effects including reductions in social play and exploration behaviour of the infant (Caldji et al., 2000). In contrast, brief handling of the neonate, a procedure that consistently increases the frequency of nest-bouts and pup care in the rat dam (Bell et al., 1971; Lee and Williams, 1974), exerts precisely opposite consequences for neuroendocrine activity: adolescents show lowered glucocorticoid secretion in response to stress (Levine et al., 1967) and are less anxious in free-exploration paradigms (Nunez et al., 1995). In general, maternal care is defined by a series of offspring-directed behavioural patterns. Lactating rats display naturally occurring inter-individual variations in the quality and quantity of attention towards their progeny, most considerably in their expression of pup-directed licking and grooming behaviour (Champagne et al., 2003). Within the normal range of maternal behaviour, mild variations in the sensory stimulation for the neonate determine individual differences in multiple developmental traits (Caldji et al., 1998; Uriarte et al., 2007). Apart from evidence for organising effects on brain regions involved in the expression of sexual behaviour (Simerly, 2002), maternal licking affects neural circuits that are implicated in the regulation of stress reactivity (Caldji et al., 1998). These effects are linked to sustained changes in the expression of genes in limbic structures that modulate hypothalamo-pituitary-adrenal (HPA) axis activity and associated behaviour (Caldji et al., 1998; Fish et al., 2004). In a study by Lui et al. (1997), strong correlations between the frequency of maternal licking/grooming and the magnitude of neuroendocrine responses to acute stress could be demonstrated. That is, subjects born to mothers who express high level of pup licking and grooming behaviour display low level of stress reactivity, whereas low licking/grooming females produce more fearful offspring with strong HPA axis responses to threat. Apart from laboratory rodents, long-term consequences of mothering styles for offspring behaviour have been documented in a variety of animal species. In primates, for instance, Altman (1980) and Fairbanks and McGuire (1988) showed that the progeny of mothers who behaved “restrictive” towards their young took longer to leave their mother for greater distances or to enter a novel environment. Hence, as the mother is the major source of stimulation during early postnatal life, neonates are highly receptive to physiological and behavioural changes in the dam (D’Amato et al., 1998) which may lead to life-long influences on behaviour and neuroendocrine activity (Cirulli et al., 2003).

In mammals, the costs of care provided for the offspring after birth, primarily lactation, is generally much greater than the energetic costs of gestation (Drent and Daan, 1980) with females often working at the limits of their capacity (Drent and Daan, 1980; Clutton-Brock et al., 1989). As maternal resources are limited, the expense of time and energy invested into current reproduction has not only consequences for subsequent fertility but also the probability of the female’s future survival (Hanwell and Peaker, 1977; Clutton-Brock et al., 1983; Neuhaus, 2000). High maternal investment increases offspring quality; however, in highly adverse environments with increased levels of physical demand and inadequate access to resources, the optimal extent of maternal effort is limited by self-maintenance (Mauck and Grubb, 1995). Reduced pup-directed activities were therefore argued to serve as a predictive signal of the concurrent environmental condition to the offspring (Meaney, 2001; Coall and Chisholm, 2003; Cameron et al., 2005) and appear to transmit or contribute to programming effects of prenatal stress (Baker et al., 2008). Even though the findings are not consistent across studies (Herrenkohl and Whitney, 1976; Fameli et al., 1995; Pardon et al., 2000; Poltyrev and Weinstock, 2005), impairment of maternal care-giving following conditions of stress during pregnancy has been assessed in the rat (Patin et al., 2002; Smith et al., 2004; Baker et al., 2008), mice (Meek et al., 2001) and primates (Rosenblum and Andrews, 1994; Lyons et al., 1998), including perturbations in mother-child attachment in humans (e.g. Whipple and Webster-Stratton, 1991). The most revealing data in support of the postnatal modulation theory emerges from cross-fostering studies in the rat, as influences of the prenatal condition were suppressed in the biological offspring of dams subjected to stress in pregnancy when raised by undisturbed females after birth (Maccari et al., 1995). So, the level of glucocorticoid response to stress was similar between adopted, i.e. prenatally stressed, and normal offspring of dams. Similarly in a study by Wakshlak and Weinstock (1990), short-term handling of the neonate reversed heightened emotional reactivity induced by prenatal stress, supposedly through alterations in dam-pup interactions engendered by this manipulation. Champagne and Meaney (2006) showed that female offspring of rats exposed to physical restraint during gestation displayed increased fear responses when adult. Most interestingly, changes in the behavioural phenotype were also apparent in the offspring of a second litter even in the absence of stress exposure during the subsequent pregnancy. Maternal influences were consequently not linked to physiological repercussions on the fetus, but were presumably caused by disruptions in the postnatal stimulation as the amount of pup licking was found chronically depressed in dams. Moreover, Champagne and Meaney (2006) were able to demonstrate that prenatal stressed female individuals behaved towards their offspring in a manner consistent with the behaviour of their mothers. The phenomenon of intergenerational transmission of parental behaviours had earlier been described by Francis et al. (1999). Hence, rat dams maintain patterns of maternal behaviour similar to those experienced early in life. Being a critical determinant for the expression of multiple phenotypic variables, it implies that selected personality traits are transmitted from one generation into the next by environmentally-driven variations in maternal care (Meaney, 2001; Fish et al., 2004).

Taken together, the extent of time and energy invested into raising young strongly depends upon the prevailing environmental circumstances and maternal condition so as to maximise individual fitness (Williams, 1966; Trivers, 1972; Clutton-Brock, 1991). Best described in the rat model, the intensity of pup-directed activities by the mother is, in turn, variously involved in offspring development, including brain and tissue differentiation processes (Cameron et al., 2005). Hypotheses by Meaney (2001), among others, rest on the assumption that exposure of the pregnant animal to demanding environmental situations sets developmental adaptations on the basis of variation in the early care-giving. Moreover, manipulations of maternal behaviour allow for the incorporation of environmental cues into the phenotypes of succeeding generations (“maternal effects”) (Champagne and Meaney, 2000).

The experimental animal used in the present study is the domestic guinea pig (*Cavia aperea f. porcellus*). It is a convenient model since influences of maternal social stress and non-social stimuli on offspring behaviour and endocrine systems have been widely described. Current findings include behavioural masculinisation and elevated plasma levels of testosterone in female offspring of dams exposed to social instabilities over the late gestational period (Kaiser et al., 2003), while short periods of nutrition deprivation and strobe light exposure in pregnancy have been shown to result in modified offspring HPA axis function and increased anxiety in juveniles respectively (Lingas et al., 1999; Kapoor and Matthews, 2005). However, the relationship between demanding environmental conditions experienced by the mother and pup care and the implication of the latter for developmental programming has not been examined yet, even though there is growing evidence from laboratory rodents other than the guinea pig. The inattention towards the early infant period might be due to the highly precocial state of guinea pig young, which contrasts caviomorphs from most mammalian taxa. As a consequence of the advanced maturity from birth, the nutritional dependence on maternal milk is reduced and care-giving by the mother only minimally expressed (Harper, 1976). Even so, the lactation period is energetically highly demanding for the guinea pig female (Mepham and Beck, 1973) and maternal milk production is substantial for the determination of pup growth early in lactation (Weaver et al., 1988; Künkele und Trillmich, 1997). Künkele and Trillmich (1997) reported infant body weight significantly correlated to maternal food intake and therefore presumably milk production, more than by the food intake of the young themselves. Behaviourally, a casual bond of attachment between the mother and offspring has been proposed by Rood (1972), as guinea pig pups will socially interact and suckle from females other than their mother. However, Porter et al. (1973) described the presence of the mother as a "secure base" for the infant from which they can explore their environment; hence, the mother appears to be of importance for the ability of the young to cope with novelty (Ritchey and Hennessy, 1987). In a series of experiments, Hennessy et al. (1995, 2000, 2002a,b) demonstrated that short periods of maternal separation evoked endocrine stress responses in the guinea pig infant even if familiar littermates are present. It is thus reasonable to assume that some form of mother-pup tie exists and cues by the mother affect neurophysiological processes and maturation in a pervasive manner (Hennessy, 2003).

The aim of the present study was to evaluate the consequences of early gestational stress experience on maternal performance, both in terms of behavioural mother-pup interactions and maternal lactational effort, and to discuss its role as mediator of environmental adversity on long-term development in the offspring. Most of the research on prenatal stress deals with clinical questions and involves the subjection to stressors at specific periods of fetal brain development, primarily during the period of greatest growth in late gestation. A different approach was preferred in the current work with stress procedures covering mating and the first and second trimester of pregnancy. To complete the data, infant body weight growth and behavioural stress responsiveness, as one of the parameters of the HPA functional development (Nishio et al., 2006), were recorded in the offspring.

I hypothesized that (1) exposure to challenging conditions during pregnancy negatively influences maternal performance postpartum; (2) potential effects of early life stress are mediated through interactions between the mother and her young.

Pup-directed activities of gestational treated and undisturbed mothers were monitored by regularly observations from birth until weaning. To determine maternal milk production, differences in infant body weights before and after suckling were registered, a method that is established to accurately estimate the amount of milk transfer (Weaver and Roberts, 1990). Fear reactivity tests were employed to assess the potential influence of maternal signals on neural development and subsequent function of offspring HPA axis. As in the rat, where subjects displaying strong HPA responses show more freezing and diminished expression of locomotor activity under conditions of adversity (Poltyrev et al., 1996; Vallee et al., 1997; Smith et al., 2004), individuals would be expected to behave less active in response to strobe light exposure. As longer latencies to eat as well as more defecation in an open-field test have been associated with an enhanced fearfulness (Weinstock et al., 1992; Caldji et al., 1998), the amount of consumed food and the occurrence of defecation were assumed to be indicative of individual stress reactivity.

Materials and Methods

Animals and housing conditions

In this experiment, short-haired, multicoloured domestic guinea pigs (*Cavia aperea* f. *porcellus*) were used. Fourteen female and seven male F0-subjects were randomly assigned to treatment or control group. Body weight of individuals did not differ significantly between groups (Mean ± SEM, female treatment subjects: 734.29 ± 29.11 g, n=7, female control subjects: 720.92 ± 31.72 g, n=7, Student's t-test, T=0.310, p=0.762; male treatment subjects: 911.81 ± 61.29 g, n=3, male control subjects: 965.5 ± 53.64 g, n=4, Student's t-test, T=-0.654, p=0.542). Except for three females, two contributed to treatment group, all dams were primiparous.

From three months prior to beginning of the experimental procedures all individuals were housed in the same room under standardised conditions with an average room temperature of 22.9 ± 0.7 °C, a relative humidity of 48 % and a 12:12 h light-dark cycle with lights on at 7.00 h. Except for stress applications, all experiments were carried out in the maintenance room.

Female subjects were kept in single-sex groups of two until mating at approximately six months of age. For this purpose, a male was introduced to female's cages and left throughout pregnancy. About three days before the expected delivery, females were removed from their groups and housed individually in maternity cages (85 x 47 cm) until the end of lactation. After weaning at the age of 21 days, pups were kept in single-sex groups of two to four separated in offspring of treated and control mothers.

Water and hay were available *ad libitum*. Daily, all individuals received 40g of fresh food (vegetables or fruits), and 15g of pelletized food for guinea pigs (Altromin 3123, Altromin GmbH, Lage, Germany). From day 45 of gestation until the end of lactation females were given an additional 20g of food pellets. Until weaning, pups received 10g of fresh food and 10g of pelletized food.

All cages were bedded with wood chips and cage cleaning took place once a week.

Stress procedure in F0-individuals

Females assigned to treatment group (n=7) were stressed using high-frequency strobe light (Mini-Flash DK 0-11, distributed by Conrad Electronic, Austria), which has previously been described to evoke a robust HPA-response in pregnant guinea pigs (Cadet et al., 1986; Kapoor and Matthews, 2005). During stress applications, females were kept individually in boxes (42 x 26 x 15 cm, LWH) containing woodchip bedding. The strobe light was positioned at a distance of about one metre above the boxes and the room was darkened during exposure. Females were submitted to stress procedures for 3 h in two blocks per day, from 9.00 to 11.00 h and 16.00 to 17.00 h, on day 7 prior to mating (day -7), day of mating (day 0 of gestation) and day 7, 14, 21, 28, 35 and 42 of gestation (gestation length= ~68 days). To determine day 7 before mating, cycle lengths of females had been recorded previous of the experimental procedures by daily inspection of the condition of the vaginal membrane. In the guinea pig, an epithelian membrane closes the vagina orifice during anoestrus and opens at the time of oestrus (Touma et al., 2001). Individuals were first stressed seven days before calculated onset of oestrus. Successful mating at time of oestrus could be estimated by the presence of a vaginal plug; otherwise the first day of full opening of the vaginal membrane was recorded as day of conception.

Measures in F0-generation

Maternal behaviour

Gestationally stressed as well as control mothers and their offspring were filmed in their maternity cages on postnatal days 1, 3, 6, 14 and 21 in two 45-minutes observation periods once in the morning, between 7.30 and 9.00 h, and once in the afternoon, between 14.00 and 15.30 h. At least four hours passed between birth of pups and first filming on day 1 postnatal. Filming usually started in the morning, except in the case of females giving birth between 3.30 and 10.00 h which were first observed in the afternoon of the day of parturition and in the morning of the following day. Thirty minutes prior to filming, the shelter hut, which was provided in every cage, was removed and the cage was placed on the floor of the maintenance room to allow filming from above. Videos were recorded and further analysed using the software program “The Observer 2.0” (Noldus Information Technology bv, Wageningen, the Netherlands). Behaviour of the mother was measured by “continuous recording” (after Altman, 1974). In addition, behavioural elements of pups that could be relevant for the interpretation of maternal behaviour were registered.

Duration or frequencies of following parameters of maternal behaviour (in Rood, 1972; Schiml and Hennessy, 1990; Hennessy and Jenkins, 1994; Albers et al., 1999a) were recorded:

- grooming: the mother exhibits nibbling or licking movements with her mouth on the face or body of an offspring (in seconds)
- anogenital licking: maternal licking of the anogenital region of the young in order to stimulate urination and defecation (in seconds)

Nursing was divided into:

  - crouching: the mother is displaying a flexed ventrum and arched back with the front legs extended to enable access to the teats (in seconds)
  - adopting a passive posture (e.g. lying beside the pups) while nursing (in seconds)
- physical contact between the bodies of the mother and one or more pups (in seconds)
- aggressive behaviour: summation of pushing, boxing and causing flight by the mother (frequency in 90 min)
- leaving pup(s): the mother moves away from her pup(s) so that distance becomes more than one body length (frequency in 90 min)
- approaching pup(s): the mother approaches pup(s) out of a distance of more than one body length (frequency in 90 min)

Following parameters were measured on pups:

- Pup leaves mother (frequency in 90 min)
- Pup approaches mother (frequency in 90 min)

Two subjects were excluded from measures of maternal behaviour and lactation performance as one female was accidently mated postpartum and due to missing records. Consequently, these data were obtained from six individuals per group.

Lactation performance

Milk yield of gestationally stressed and control females was measured using a method described by Laurien-Kehnen and Trillmich, 2003 (modified after Mepham and Beck, 1973). On postnatal days 4, 8 and 12 the litter was separated from their mother for 1.5 hours, from 11.15 to 12.45 h, during which they were kept in bedded boxes without access to water and food. Body weight of each pup was then determined to the nearest of 0.01 g (using RS 232, Radwag GmbH, Hilden, Germany) and pups were placed back into the maternity cage, from which water and food had been removed earlier. During the following period of 30 minutes, the duration of suckling of individual pups was continuously registered by an experimenter present in the maintenance room. Suckling was recorded when a pup was positioned such that its snout contacted its mother’s ventrum for longer than 30 seconds and milk could be drunk (in Schiml and Hennessy, 1990).

At the end of this period, pups were removed and their body weights measured again. Differences in the individual body weights were interpreted as the amount of milk yield per pup.

Measures in F1-generation

Pup growth

Within 15 minutes after parturition, birth weights of pups were registered. For determination of growth rates, infant body weights were monitored daily at 9.00 h throughout the lactation period, i.e. 21 days following birth.

Behavioural response to stress

To access behavioural stress responsiveness in the adult offspring, F1-subjects were exposed to strobe light using the same method as described above (Stress procedure in F0-individuals). Again, stress procedures were applied in two trials per day, between 9.00 and 11.00 h as well as 16.00 and 17.00 h. Testing was carried out on day 8 of the 4th oestrus cycle, i.e. the 4th cycle that followed the onset of puberty, in female offspring. Male offspring were tested together with a female subject of the same treatment group. Age at time of testing ranged between day 84 and 148 of life with mean age not differing significantly between groups (Mean ± SEM, prenatal treated offspring: 104 ± 3.09 days, n=20, control offspring: 104.92 ± 2.75 days, n=25, Mann-Whitney U-test, U=242, p=0.855).

During exposure, food (vegetables or fruits), each 20g in the morning and afternoon session, were available inside the testing boxes. I measured how much individuals had fed during stress application by weighing the remained food ration subsequent to each test and subtracting the amount from the original. Further, the occurrence of defecation during testing was registered by looking for faeces in the boxes at the end of each trial.

The behaviour of individuals was recorded for the first 10 minutes of each testing trial. For filming, a video camera was positioned above the boxes whereby no additional lighting was used. Taped video films were analysed using "The Observer 2.0".

Measured behavioural parameters on individuals were:
- Latency to move: first position change of individuals by moving with the whole of the body (in seconds)
- Occurrence of locomotor activity: movements of one or more body lengths distance (yes/no)
- Occurrence of eating food (yes/no)

With the exception of the amount of food intake, individuals were not found to differ significantly between testing trials in the morning and afternoon (*latency to move:* Wilcoxon test, n(morning/afternoon)=40/42, Z=-0.958, p=0.338; *locomotor activity:* Chi-square test, n(morning/afternoon)=40/42, χ^2=1.397, p=0.237; *eating:* Chi-square test, n(morning/afternoon)=40/40, χ^2=0.000, p=1.000; *food intake:* Wilcoxon test, n(morning/afternoon)=43/43, Z=-4.291, p<0.001; *defecation:* Chi-square test, n(morning/afternoon)=45/45, χ^2=1.540, p=0.215). Therefore analyses were not performed separately for morning and afternoon sessions (except for food consumption).

Statistics

Normal distributions of values were tested prior to the use of parametric statistics (Shapiro-Wilk test). Between-group comparisons of maternal behaviour were performed by applying repeated measurements analyses of variance (ANOVA; maternal behaviour as interaction of time) or Student's t-test (maternal behaviour for each observation day). Nursing parameters were significantly affected by litter size and litter size therefore included as covariate in statistical analyses. For individual milk yield and suckling duration, a litter mean was calculated and analysed using univariate ANOVA or repeated measurements ANOVA. For determination of differences in birth weight and body weight growth of treated and control offspring, Student's t-test and repeated measurements ANOVA were made. Female and male offspring were analysed separately when differing statistically.

To compare stress response data of treated and control offspring, Mann-Whitney U-test (latency to move and amount of food intake) and Chi-square test (occurrence of locomotor activity and occurrence of eating) were carried out.

All statistical computations were conducted using "SPPS 17.0" (SPSS Inc. for Windows, 2008, Chicago, IL, USA). Values of $p < 0.05$ were considered significant.

Results

Litter characteristics

There were no significant effects of stress treatment with regards to litter size and the offspring's sex ratio. Litter sizes ranged from 1 to 5 pups with a mean of 3.14 pups in treated and 3.57 in control litter (Student's t-test, $n_{stressed}=7$, $n_{control}=7$, T=-0.783, p=0.449). Mean percent of males relative to total number of offspring per litter were 0.54 and 0.55 respectively.

Maternal behaviour

Grooming and anogenital licking

Time and treatment affected the expression of pup-directed grooming by trend with prenatal stressed mothers tending to display more grooming compared to control ones (repeated measurements ANOVA, time: df=4/36, F=2.298, p=0.078, group: df=1/9, F=3.601, p=0.090). Grooming behaviour was not found to differ significantly between treated and control subjects over time (repeated measurements ANOVA, time x group: df=4/36**,** F=1.714, p=0.168; Fig. 1A).

A significant decline in the amount of anogenital licking towards pups could be observed over lactation; however, licking was found to be unaffected by treatment and there were no significant group differences over time (repeated measurements ANOVA, time: df=4/36, F=6.885, p<0.001, group: df=1/9, F=0.017, p=0.898, time x group: df=4/36, F=0.392, p=0.813; Fig. 1B).

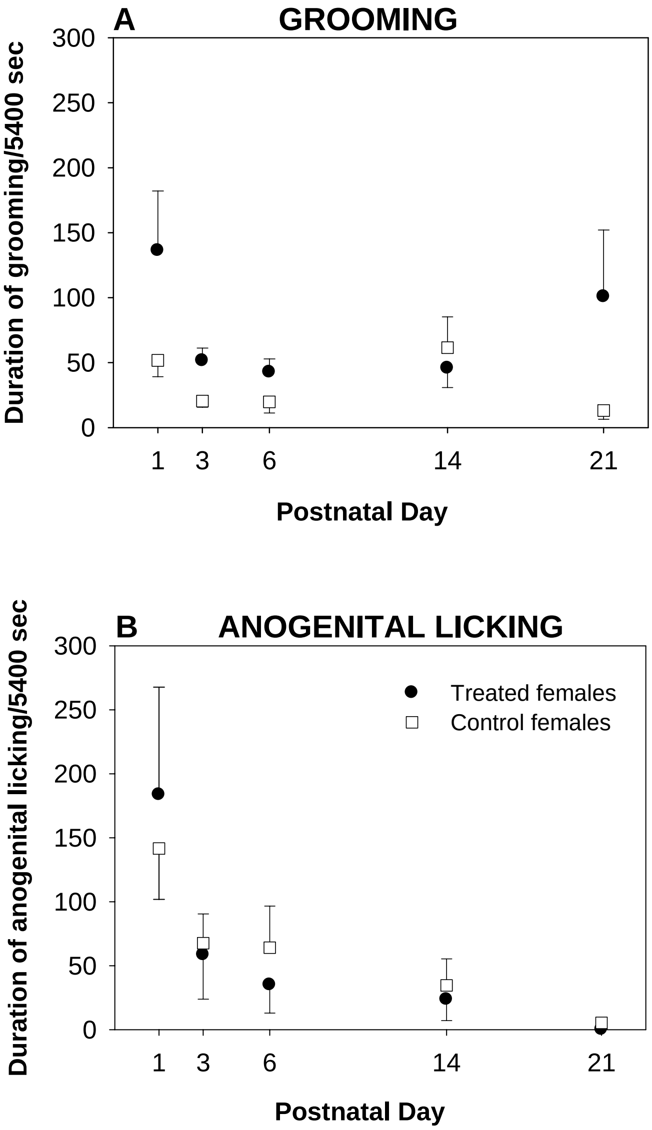


Figure 1: Duration (in seconds) of offspring-directed grooming (A) and anogenital licking (B) shown by gestationally stressed (solid circles; n=6) and control females (open squares; except day 1 (n=5): n=6) over the lactation period. Values are given as mean ± SEM.

Nursing behaviour and physical contact between mother and pups

Nursing activities were recorded as the duration of crouching or passive nursing of their young, as well as both parameters summed up as total nursing behaviour. With the exception of crouching, nursing behaviour declined significantly with time, but no group effect could be found (*crouching:* repeated measurements ANOVA, time: df=4/36, F=0.686, p=0.606, group: df=1/9, F=0.021, p=0.888; *passive nursing:* repeated measurements ANOVA, time: df=4/36, F=9.398, p<0.001, group: df=1/9, F=0.321, p=0.585; *total nursing:* repeated measurements

ANOVA, time: df=4/36, F=9.692, $p<0.001$, group: df=1/9, F=0.251, p=0.654). Nearly all comparisons revealed a higher amount of nursing in undisturbed females; however, no nursing parameter differed significantly between treated and control subjects over lactation (*crouching:* repeated measurements ANOVA, time x group: df=4/36, F=0.506, p=0.731; *passive nursing:* repeated measurements ANOVA, time x group: df=4/36, F=0.394, p=0.812; *total nursing time:* repeated measurements ANOVA, time x group: df=4/36, F=1.750, p=0.160; Fig. 2A,B, 3A).

Time, but not treatment affected the duration of physical contact between mother and pups (repeated measurements ANOVA, time: df=4/36, F=3.508, p=0.016, group: df=1/9, F=0.628, p=0.449). Even though physical contact was consistently higher in the control than in the treatment group, in a repeated measurements analysis, group differences were not significant over time (repeated measurements ANOVA, time x group: df=4/36, F=0.656, p=0.626; Fig. 3B). Nonetheless, treatment tended to affect maternal behaviour on the first day postpartum with gestationally stressed females displaying less physical contact as well as total nursing behaviour than control ones (*contact:* Student's t-test, $n_{stressed}=6$, $n_{control}=5$, T=2.052, p=0.07; *nursing:* Student's t-test, $n_{stressed}=6$, $n_{control}=5$, T=2.106, p=0.065).

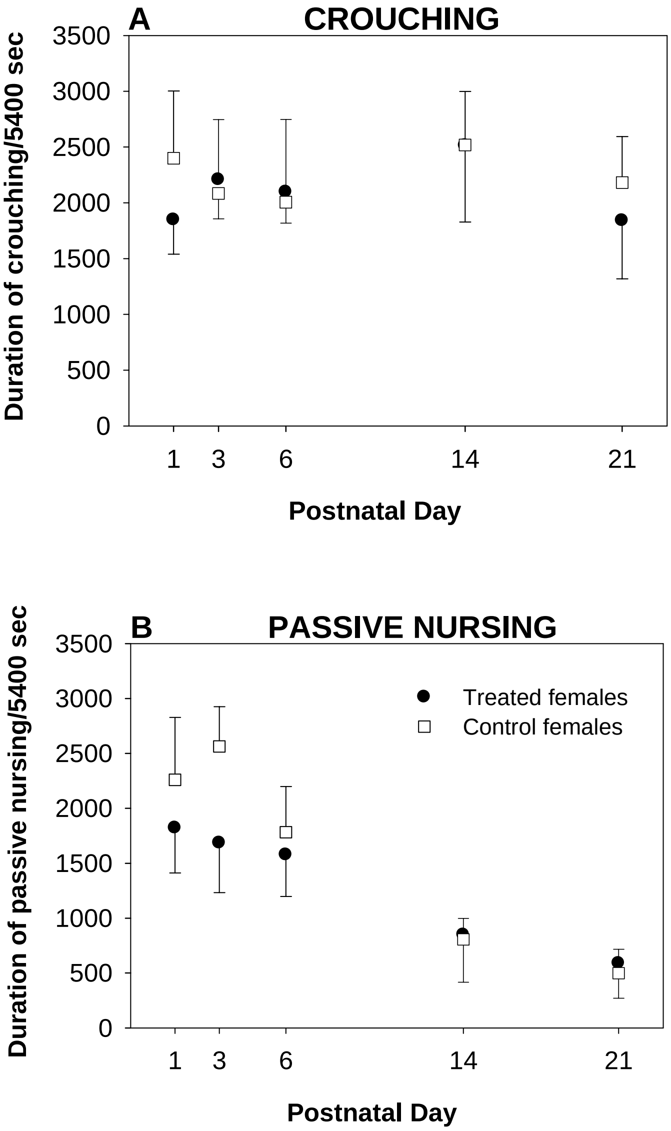


Figure 2: Duration (in seconds) of crouching (A) and passive nursing (B) in gestationally stressed (solid circles; n=6) and control females (open squares; except day 1 (n=5): n=6) over lactation. Values are given as mean ± SEM.

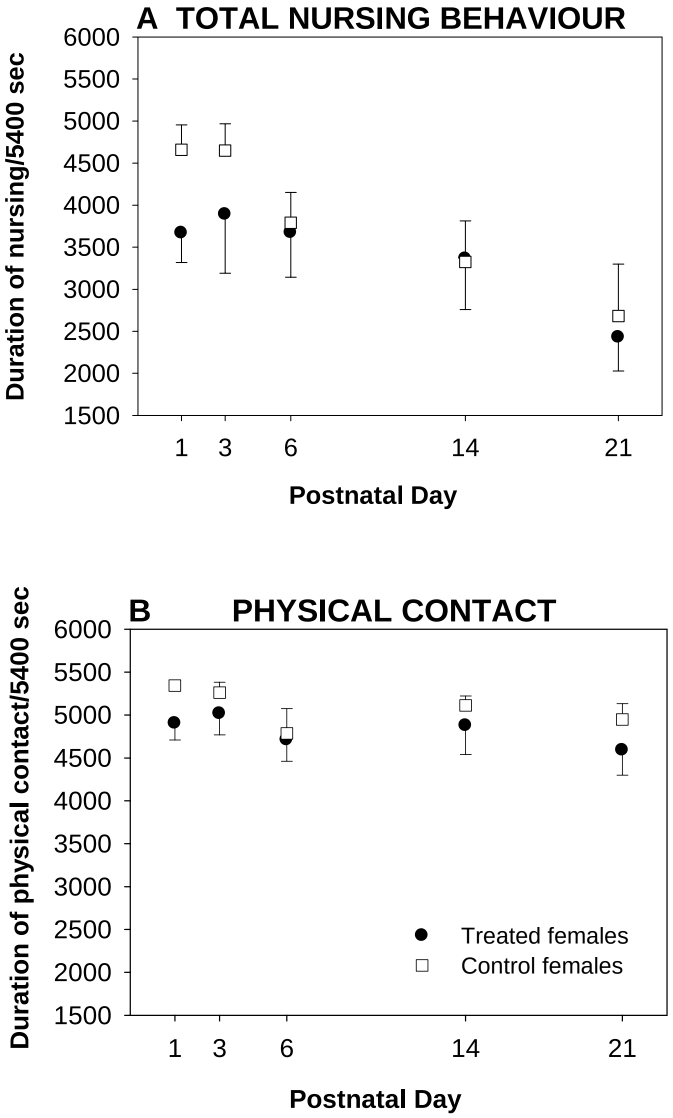


Figure 3: Duration (in seconds) of total nursing behaviour (A) and physical contact between the mother and her litter (B) in gestationally stressed (solid circles; n=6) and control females (open squares; except day 1 (n=5): n=6) over lactation. Values are given as mean ± SEM.

Aggressive behaviour and moving activities

Frequencies of offspring-directed aggression increased significantly over lactation but no group effects could be found (repeated measurements ANOVA, time: df=4/36, F=13.761, p<0.001, group: df=1/9, F=0.215, p=0.137). However, females in the stress condition developed a significantly higher level of aggression towards their pups in comparison to controls (repeated measurements ANOVA, time x group: df=4/36, F=4.237, p=0.007; Fig. 4).

There were marginally significant time but no group effects for leaving frequencies by the mother (repeated measurements ANOVA, time: df=4/36, F=2.528, p=0.057, group: df=1/9, F=1.315, p=0.281). Although gestationally stressed females left their litter at a higher rate than control ones at each observation day, no significant group variation could be found with time (repeated measurements ANOVA, group x time: df=1/36, F=0.209, p=0.932; Fig. 5A). Dams did not differ significantly in their approaching frequencies over time and there was no significant impact of treatment (repeated measurements ANOVA, time: df=4/36, F=1.904, p=0.131, group: df=1/9, F=0.239, p=0.636). Also, a repeated measurements analysis revealed no differences in their approaching frequencies over time (repeated measurements ANOVA, time x group: df=4/36, F=1.501, p=0.222; Fig.5B).

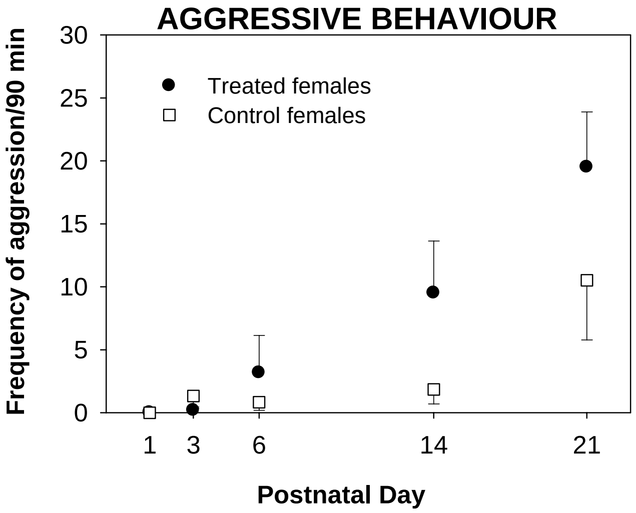


Figure 4: Frequency (in 90 minutes) of offspring-directed aggressive behaviour in gestationally stressed (solid circles; n=6) and control females (open squares; except day 1 (n=5): n=6) over lactation. Values are given as mean ± SEM.

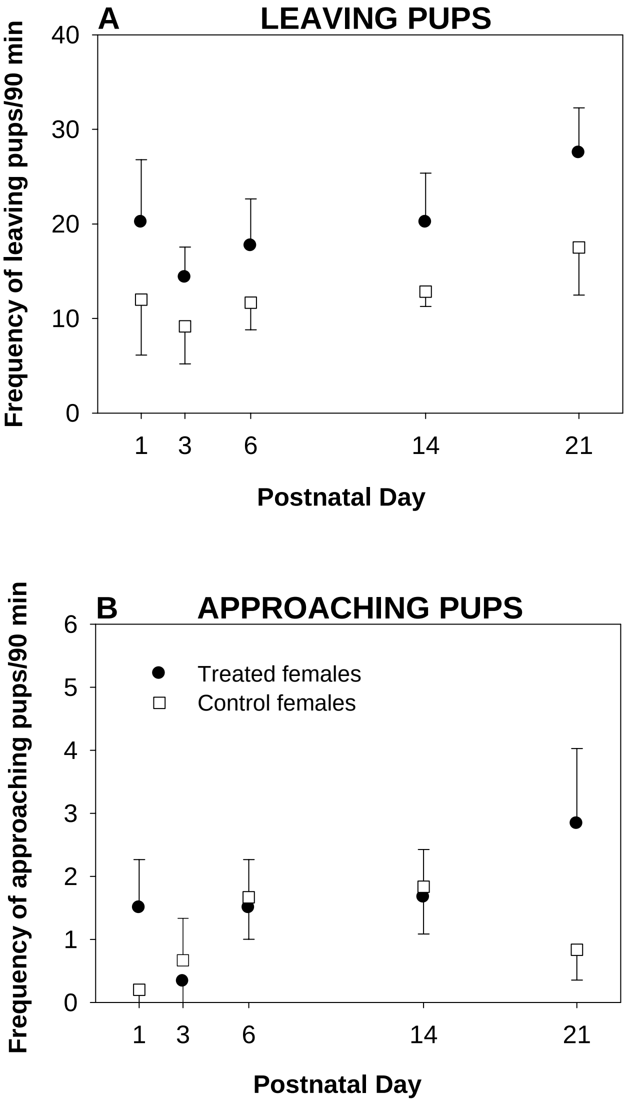


Figure 5: Frequency (in 90 minutes) of leaving (A) and approaching pups (B) in gestationally stressed (solid circles; n=6) and control females (open squares; except day 1 (n=5): n=6) over lactation. Values are given as mean ± SEM.

Moving activities of pups

Approaching frequencies of pups towards their mother were calculated per second without contact to the mother. There were no significant changes in the approaching frequency with increasing age of pups; however, offspring tended to leave their mother more often with time (*leave:* repeated measurements ANOVA, time: df=4/36, F=2.155, p=0.094; *approach:* repeated measurements ANOVA, time: df=4/36, F=0.948, p=0.447). Both parameter were unaffected by treatment and no significant interactions between time and group were found (*leave:* repeated measurements ANOVA, group: df=1/9, F=0.035, p=0.856, time x group: df=4/36, F=1.974, p=0.119; *approach:* repeated measurements ANOVA, group: df=1/9, F=0.485, p=0.502, time x group: df=4/36, F=1.212, p=0.321; Fig. 6A,B).

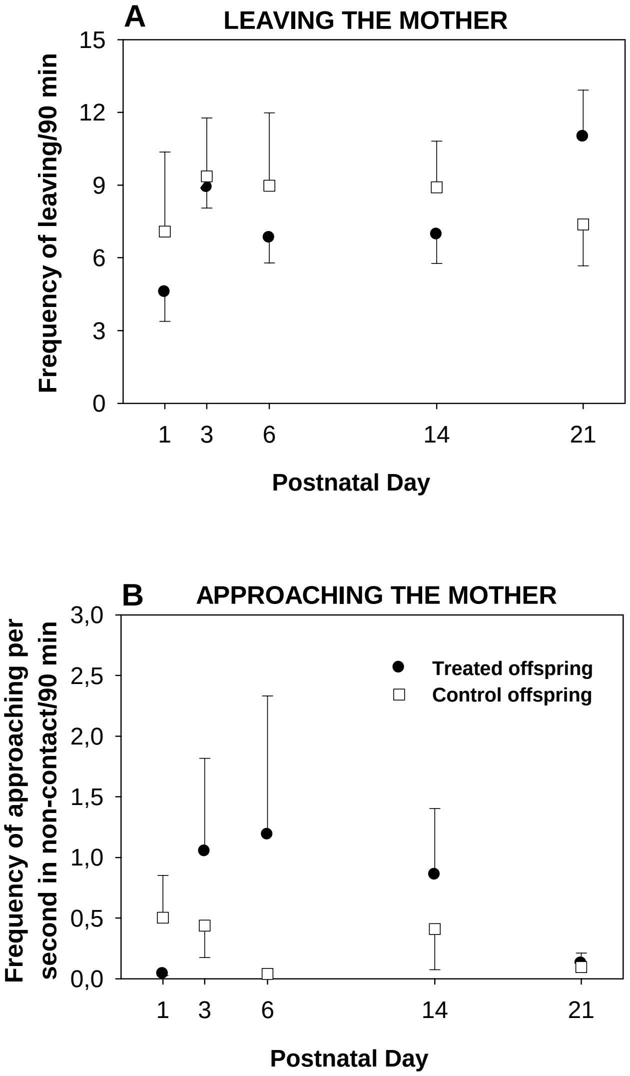
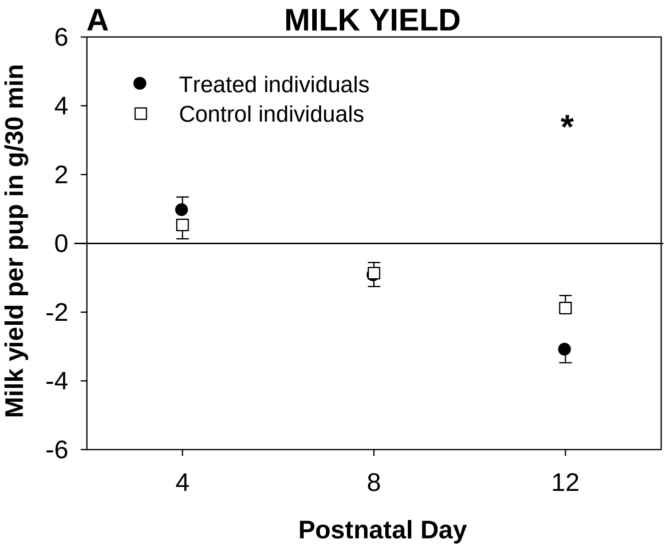


Figure 6: Frequency (in 90 minutes) of leaving the mother (A) and approaching the mother per second without contact to the mother (B) in prenatal stressed (solid circles; n=6) and control offspring (open squares; except for day 1(n=5): n=6) over the lactation period. Frequency values were calculated as mean of litter means (± SEM).

Lactation performance

Individual milk yield as well as suckling duration per pup were similar between the sexes (*milk yield:* ANOVA, df=1/18, F=0.053, p=0.821; *suckling:* ANOVA, df=1/18, F=0.258, p=0.618). Despite the fact that there was no decrease in nursing over the whole period of lactation, milk yield per pup was found to decline continuously. It has to be noted that most measures on milk yield revealed higher body weight loss than weight gain by milk intake except for lactation day four.

Marginally significant time effects, but no significant group differences were found for milk intake and suckling measures on pups. While stressed dams showed significantly lowered milk yield than controls over time, their offspring tended to suckle longer (*milk yield:* repeated measurements ANOVA, time: df=2/18, F=3.464, p=0.053, group: df=1/9, F=0.603, p=0.457, time x group: df=2/18, F=4.182, p=0.032; *suckling:* repeated measurements ANOVA, time: df=2/18, F=3.377, p=0.057, group: df=1/9, F=1.752, p=0.218, time x group: df=2/18, F=2.785, p=0.088; Fig. 7A, B). Separate day analyses revealed significant group differences at lactation day twelve with stressed offspring suckling longer than control one but a lower amount of milk being transferred (*milk yield:* ANOVA, df=1/9, day 4: F=0.531, p=0.485, day 8: F=0.034, p=0.858, day 12: F=5.695, p=0.041; *suckling:* ANOVA, df=1/9, day 4: F=0.555, p=0.475, day 8: F=0.011, p=0.920, day 12: F=6.319, p=0.033).



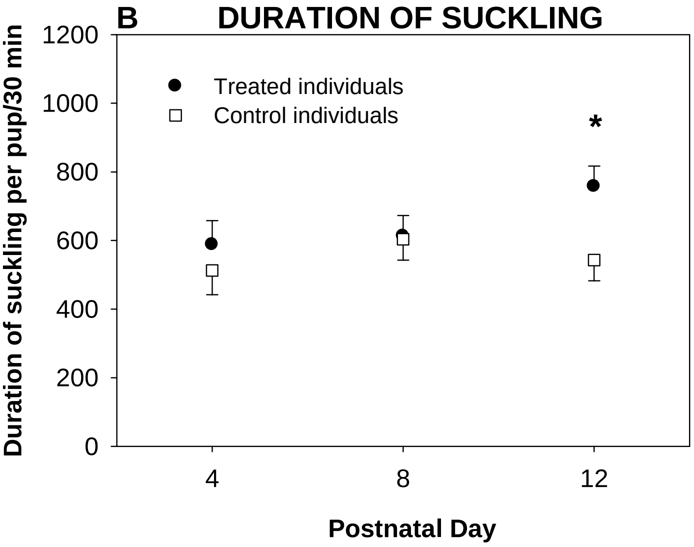
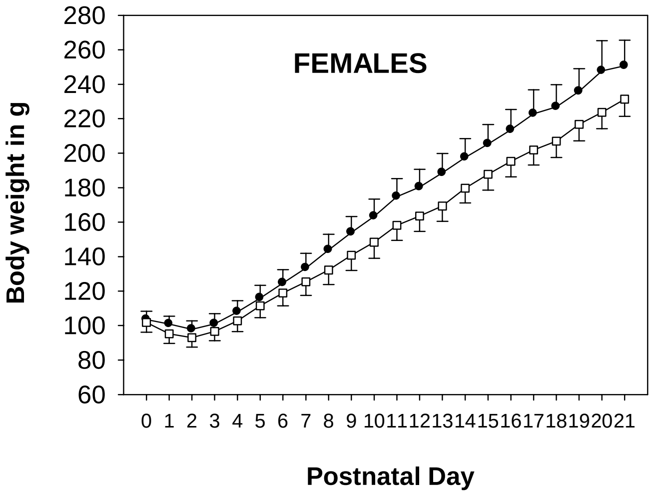


Figure 7: Individual milk yield (in g) (A) and duration of suckling (in seconds) (B) per pup in the offspring of gestationally stressed individuals (solid circles; n=6) and control subjects (open squares; n=6) during a period of 30 minutes (*****$p < 0.05$). Values given are means of litter means ± SE.

Body weight growth of pups

Body weight at birth did not differ significantly between sexes (Student's t-test, $n_{females}$=21, n_{males}=26, T=1.373, p=0.176). Nonetheless, females diverged significantly from male subjects in their body weight growth over lactation (repeated measurements ANOVA, sex: df=1/41, F=1.767, p=0.191, time: df=21/861, F=36.648, p<0.001, sex x time: df=21/861, F=22.714, p=0.013).

There was no effect of stress treatment on pup weight at birth (Mean ± SE, offspring of treated mothers: 105.4 ± 3.48 g, n=22, control offspring: 103.51 ± 3.82 g, n=25, Student's t-test, T=0.360, p=0.773). Groups were not found to differ significantly over time. However, there were stronger discrepancies in the body weight growth of female than male subjects with treated individuals showing consistently higher body weight than control ones (*females:* repeated measurements ANOVA, time: df=21/336, F=150.622, p<0.001, group: df=1/16, F=2.025, p=0.174, time x group: df=21/336, F=0.664, p=0.868; *males:* repeated measurements ANOVA, time: df=21/462, F=185.332, p<0.001, group: df=1/22, F=1.014, p=0.352, time x group: df=21/426, F=0.849, p=0.364; Fig. 8).



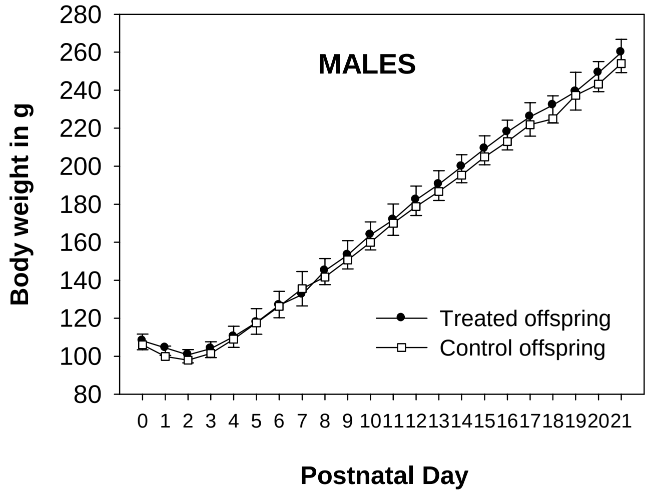


Figure 8: Body weight growth of prenatal stressed (solid circles) and control individuals (open squares) during lactation (Postnatal day 0 indicates birth weight). Numbers of animals (offspring of gestationally stressed subjects/offspring of undisturbed subjects) were 8/11 in female and 12/13 in male offspring. Values are given as mean ± SE.

Behavioural stress response in the adult offspring

Sexes did not differ significantly for any of the behavioural elements (*latency to move:* Mann-Whitney U-test, $n_{females}$=19, n_{males}=23, U=201, p=0.658; *locomotor activity:* Chi-square test, 1st trial: $n_{females}$=17, n_{males}=23, χ^2=1.085, p=0.298, 2nd trial: $n_{females}$=19, n_{males}=23, χ^2=0.766, p=0.382; *eating:* Chi-square test, 1st trial: $n_{females}$=17, n_{males}=23, χ^2=1.319, p=0.251, 2nd trial: $n_{females}$=18, n_{males}=22, χ^2=0.175, p=0.676; *food intake:* Mann-Whitney U-test, 1st trial:

$n_{females}$=19, n_{males}=24, U=224.5, p=0.931, 2nd trial: $n_{females}$=19, n_{males}=24, U=206, p=0.590; *defecation:* Chi-square test, 1st trial: $n_{females}$=20, n_{males}=25, χ^2=1.301, p=0.254, 2nd trial: $n_{females}$=20, n_{males}=25, χ^2=0.076, p=0.783). Consequently, no separation between female and male offspring was done.

No significant treatment effects were found for latency to move or the expression of locomotor activity during stress exposure. Nonetheless, there was a marginally significant group difference for the first testing trial in the morning with prenatally stressed offspring showing less locomotor activity compared to control one (*latency to move:* Mann-Whitney U-test, $n_{treated}$=20, $n_{control}$=22, U=208, p=0.762; *locomotor activity:* Chi-square test, 1st trial: $n_{treated}$=19, $n_{control}$=21, χ^2=3.647, p=0.056, 2nd trial: $n_{treated}$=20, $n_{control}$=22, χ^2=0.001, p=0.976; Fig. 9A,B).

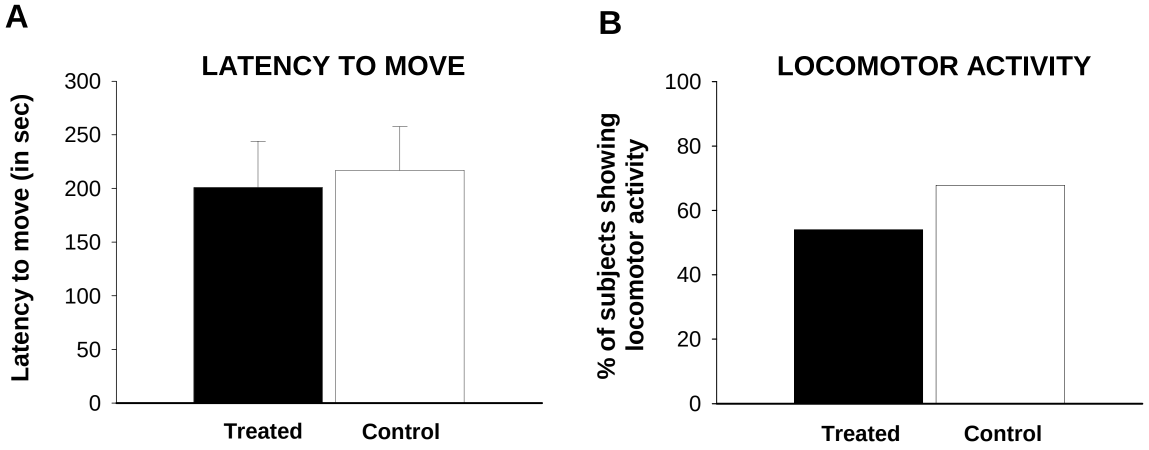


Figure 9: Latency to move (mean ± SEM) (A) and mean percent of subjects displaying locomotor activity (B) in prenatal stressed and control offspring during the first 10 minutes of exposure to strobe light. Numbers of animals (in the morning/afternoon trial) were 19/20 in treated and 21/22 in control offspring.

There were no significant differences between offspring of gestationally stressed and control mothers in the occurrence of eating (Chi-square test, 1st trial: $n_{treated}$=19, $n_{control}$=21, χ^2=1.520, p=0.281, 2nd trial: $n_{treated}$=20, $n_{control}$=20, χ^2=0.102, p=0.749; Fig. 10A). Both prenatal stressed and control individuals were observed to eat significantly less during the second stress exposure compared to the first trial in the morning. Although subjects of treated and control group did not differ significantly in their food intake, there was a tendency towards a lower amount of food consumption in prenatal stressed offspring during the morning session, indicating less activity during the whole of the stress exposure (Mann-Whitney U-test, 1st trial: $n_{treated}$=20, $n_{control}$=23, U=154, p=0.060, 2nd trial: $n_{treated}$=20, $n_{control}$=23, U=211.5, p=0.652; Fig. 10B).

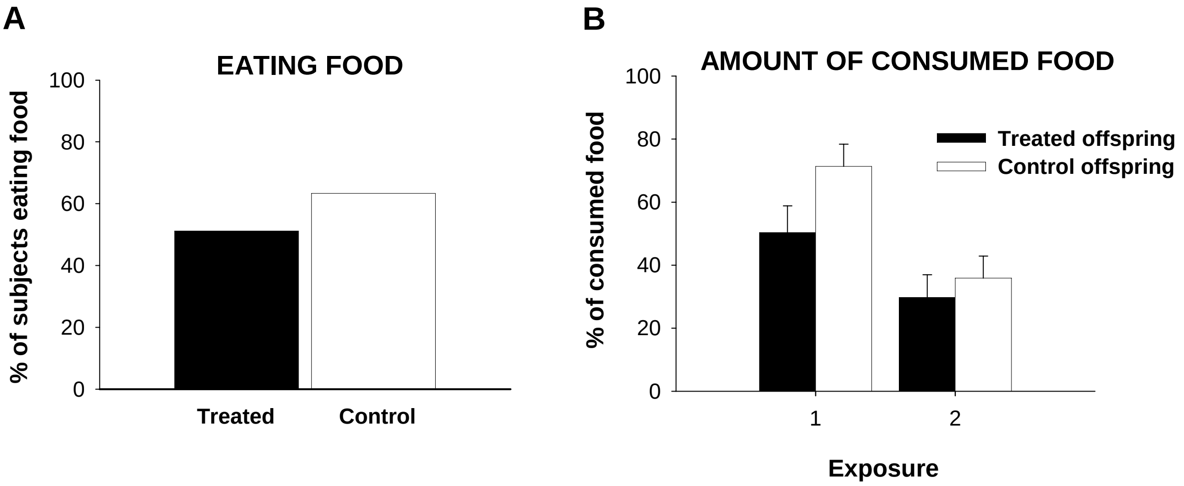


Figure 10: Mean percent of treated and control subjects observed to eat during stress exposure (A). Percent of consumed food (mean ± SEM) by prenatal stressed and control individuals during the 1st and 2nd exposure to strobe light (B). Numbers of animals (in the morning/afternoon trial) were 19/20 in treated and 21/20 in control offspring for measures on the occurrence of eating and 20/20 in treated and 23/23 in control offspring for the amount of food intake.

Treatment and control group were not found to differ significantly in the number of individuals defecating during stress exposure (morning: 10/20 in treated offspring, 15/25 in control offspring; afternoon: 9/20 in treated offspring, 8/25 in control offspring; Chi-square test, 1st trial: $n_{treated}$=20, $n_{control}$=25, χ^2=0.450, p=0.502, 2nd trial: $n_{treated}$=20, $n_{control}$=25, χ^2=0.799, p=0.371).

Discussion

This study was designed to evaluate effects of early gestational stress exposure in the mother as well as offspring in the guinea pig model. Focus was on long-term consequences of stress experience in the mother and I addressed the question whether alterations in maternal behaviour and lactational effort might contribute to developmental changes observed in the progeny.

Maternal behaviour

The preceding gestational treatment had no significant effect on the expression of pup-directed grooming and licking behaviour; however, there was a tendency towards increased grooming in mothers of the stress condition. This is in some dissimilarity to prior assessments of depressed grooming and licking behaviour in stressed rat dams (Moore and Power, 1986; Patin et al., 2002; Champagne and Meaney, 2000). For instance, Baker et al. (2008) showed that rats exposed to physical restraint in late pregnancy spent less time engaged in pup care, primarily caused by impairments in the amount of pup licking. Complementary to their study, Champagne and Meaney (2006) reported that rat dams were individually affected by gestational stress experience. That is, only females previously described as high grooming/licking mothers expressed diminished levels after exposure to physical restraint in a second pregnancy, while dams displaying low amounts of grooming and licking towards their pups *per se* were unaffected by stress treatment. Female guinea pigs have been found to vary greatly in their mothering styles (Albers et al., 1999b); hence, it might be difficult to assess behavioural alterations as result of environmental manipulations. This applies in particular to relatively small sample sizes, such as the one that was used in my study. It is, however, not clear whether grooming and licking behaviour by the guinea pig dam relates to the same developmental consequences as documented in the rat neonate. Licking of the infant’s fur and anogenital region is only minimally expressed with guinea pig young being able to clean themselves shortly after birth (Coulon, 1971; Fullerton et al., 1973). The importance of these behavioural patterns for offspring maturation processes has yet to be addressed in a systematic way in the guinea pig.

In rodents, dams are observed to display grooming and anogenital licking behaviour mostly during the active form of nursing, in an arched-back position, also referred to as crouching (Caldji et al., 2000, Fish et al., 2004). This is not the only posture from which they nurse, as females are also observed to take up more relaxed positions, e.g. lying on their side (Fish et al., 2004), which often diminishes the opportunity of more than one pup to suckle. In the present study, neither crouching nor passive nursing were significantly affected by gestational stress treatment. Nonetheless, the total time spent nursing was slightly decreased at a single day, the day following birth, which supposedly correlated with the fact that stressed dams spent more time absent from pups. Stress experienced females were observed to engage not significantly but constantly less time in social contact to their offspring than undisturbed mothers over the period of lactation. Despite well documented effects on the expression of grooming/licking behaviour, only few studies were consistent in their findings of modified nursing behaviour in stressed dams. Not all authors separated between nursing in the arched-back position and passive nursing. While Smith et al. (2004) reported diminished arched-back nursing in stressed rats, nursing was not found affected by others (Meek et al., 2001; Patin et al., 2002; Champagne and Meaney, 2006). Influences of environmental conditions on the overall time spent in physical contact to pups, e.g. time spent in the nest with the litter, has not been documented yet in laboratory rodents (Muir et al., 1985; Moore and Power, 1986; Meek et al., 2001; Smith et al., 2004; Champagne and Meaney, 2006). It has to be taken into consideration that the presence of the mother is essential for survival in the rat and mice, as dams have to keep constant body temperature of their young by hovering over them. In contrast, guinea pig pups are endothermic from birth and do not necessarily require the constant presence of the mother (Gelineo, 1964).

Gestational stress did not result in distinct differences of the dam’s moving activities in my study. However, stressed females were tendencially more likely to leave their litter in order to eat or drink, affecting the time females spent in contact to pups. In accordance, Patin et al. (2002) found enhanced self-directed activities in prenatal stressed rats, including resting or eating, proposing that females took more care of themselves than of their offspring. In the present work, the stable decreased physical contact to pups, taken together with significantly enhanced maternal aggressiveness suggests that stressed guinea pig dams were less responsive towards their infants than control ones. Pardon et al. (2000) showed that chronic gestational stress exposure impaired the ability of lactating mice to protect their offspring against an intruder male. That is, females allowed the male to attack their pups as they were lacking any hostile behaviour towards the intruder. These results were linked to disruptions in the mother/pup signaling, disabling the mother to respond accurately to their offspring’s need (Pardon et al., 2000). Supported by reports of greatly impaired retrieving latencies in stressed rats (Patin et al., 2002), this might explain why stressed guinea pig mothers behaved more aggressive towards their progeny. Moreover, experiences of stressful situations in gestation have been associated with changes in the rat female’s emotional reactivity, reflected in symptoms related to anxiety and depressive-like behaviour lasting well beyond the period of actual exposure (Darnaudery et al., 2004; Smith et al., 2004). This state of increased fearfulness has been hypothesized to perturb care-giving by the mother (Smith et al., 2004) and may also facilitate adverse behaviour towards the young.

Nonetheless, it has to be questioned whether effects on the mother-offspring interaction were due to behavioural changes in the mother or to a lack of demand from their young. In fact, as the pup's affiliative behaviour is, at least in part, a determinant for competence of maternal care (Fleming et al., 1994; Morgan et al., 1994), alterations in the offspring's behaviour have been proposed as possible cue for impairments in care-taking of mothers stressed in pregnancy (Meek et al., 2001). In support of this idea are findings that none-stressed rat dams raising cross-fostered, prenatally stressed pups were less likely to groom and nurse their progeny than mothers raising adopted, undisturbed pups (Moore and Power, 1986; Meek et al., 2001). In addition, the precociality of guinea pig young enables them to initiate interactions with the mother (Fey and Trillmich, 2007); hence, moving activities of pups could be a contributing factor for maternal care. Even though no significant variations in the activity of prenatal stressed and control offspring could be verified in my data, infants of stressed dams were observed to approach their mother at tendencially higher rates than controls, indicating that pups reacted accurately to the female's leaving activities. This implies that the observed impairments in the mother-pup relation, as far as physical contact is concerned, were supposedly not caused by alterations in the pup's attachment behaviour towards the mother.

To sum up, some parameters of maternal behaviour were found influenced by early gestational stress experience, but effects were for the most part of a subtle nature. Nonetheless, it is yet unclear if and to what extent environmental factors impact on the dam-offspring relationship in the guinea pig, a species in which maternal care is described as being not very pronounced (Kunkel and Kunkel, 1964; Harper, 1976). Hence, the slightly decrease in nursing and physical contact to pups may represent a substantial modification in maternal physiology, which is supported by my findings of significantly altered aggressive behaviour. As previous studies applied stress paradigms in general over the mid to late gestational period, it is plausible that behavioural consequences for the mother may depend on the stage of gestation covered by experimental treatment, even though Patin et al. (2002) demonstrated significant alterations in pup-directed activities in the rat irrespective of whether the stressor was administrated at day 10 or 14 of gestation (gestational length=21 days). There again, the delay between last stress application and parturition might contribute to the only modest effects observed. Compared to two weeks, at the outmost, in research on the rat and mice, more than three weeks passed between these events in the present work.

The results obtained from my study were conflicting as gestational stress exposure negatively affected the expression of some behavioural elements of pup care, while a trend towards enhanced grooming was evident. Pup grooming and anogenital licking behaviour of the lactating female are of particular interest for research on prenatal stress effects with regards to their proposed role for programming changes of HPA axis function and fear related behaviour in rodents (Lui et al., 1997; Francis et al., 1999). On the one hand, stress-dependent changes in grooming/licking are difficult to assess as individuals vary in their behaviour even in the absence of experimental manipulations (Albers et al., 1999a; Fish et al., 2004). However, my data may also raise the question of the relevance of nurturing behaviour for infant central nervous system (CNS) maturation in the guinea pig. Analyses linking maternal grooming and licking with organising effects on the development of HPA axis function are derived from research on the rat, i.e. a species in which a critical period of neuroendocrine development occurs during the preweaning period (Sapolsky and Meaney, 1986). In contrast, maximal brain growth and a considerable amount of neural maturation take place prenatally in the guinea pig (Dobbing and Sands, 1970, 1979; Jones and Roebuck, 1980). Maternal behaviour will thus affect different stages of offspring development as a function of the species studied. As a result of the high degree of maturity at birth (Weinstock, 2005) the potential for phenotypic plasticity through manipulations in early maternal care-taking is arguable in the guinea pig. Moreover, Macri and Würbel (2006) predicted that similar developmental adjustments to adverse environments might be due to species-specific maternal and/or environmental signals transmitted to the offspring. Experimental work on maternal programming is to a great part based on the rat and mice. In these altricial species, pups have little direct experience with the environment prior to weaning as they spend their first weeks of life in a safe and stable nest (Cadji et al., 2000). Hence, the mother is apart from littermates the most relevant source of sensory stimulation for the neonate (Francis and Meaney, 1999) and the lactating female may then serve to transmit information pertaining to the environment through variations in the characteristics of pup care (Caldji et al., 2000). However, the guinea pig stands out from other rodents by producing extremely precocious young that are able to locomote from their first day of life (Kunkel and Kunkel, 1964) and have to cope with environmental contingencies at an early age (Porter et al., 1973). Infants therefore do not need to rely on maternal behaviour as an indirect signal of future life conditions; in addition, the fact that guinea pig pups require little active care from the mother (Kunkel and Kunkel, 1964; Fullerton et al., 1973) suggests a reduced sensitivity to maternal stimuli in the postpartum developmental period. In support are findings by Parker et al. (2006) that stress resistance in squirrel monkeys varied with environmental stress independently of maternal care. Macri and Würbel (2006) related this to the fact that in primates, females tend to carry their young with them and infants are exposed to their environmental situation immediately after birth.

Lactational performance and offspring weight development

In agreement with my expectations, stress conditions during pregnancy resulted in diminished lactational effort by the dam. Interestingly, effects on maternal milk secretion emerged as an interaction of time and coincided with tendencially longer suckling durations in prenatal stressed pups. This might indicate a heightened demand by the offspring; however, cross-fostering experiments suggest that guinea pig dams do not adjust their milk secretion to the body condition of their young or the intensity of suckling stimulation (Laurien-Kehnen and Trillmich, 2003; Rehling and Trillmich, 2008).

Even though behavioural impairments in the female nursing performance have been previously found in the rat (e.g. Smith et al., 2004), only a limited number of studies have yet evaluated consequences of gestational stress for milk production or quality directly. Nonetheless, Lau (1992) reported reduced milk release in rats treated by various stressors in pregnancy and suspected a hormonal-directed suppression of milk synthesis or ejection. In the present study, depressed maternal milk yield was accompanied by heightened pup-directed aggressiveness in females, which in general were refuses of suckling attempts. This can be considered as a strategy to make suckling unprofitable for the young, which then contribute to their energy requirement with increased self-feeding (Rehling and Trillmich, 2007). Such a proceeding might, in turn, accelerate the process of weaning. In accordance are findings by Arnold et al. (1979) and Lee (1984) that under highly adverse conditions in stressful environments, females tended to wean their offspring early.

In the guinea pig, the highest milk yield is usually detected around day 7 of lactation (Mepham and Beck, 1973; Anderson et al., 1984; Laurien-Kehnen and Trillmich, 2003). No such yield curve was assessed in the present study as weight losses of pups were higher than milk intake at lactational days 8 and 12. For this reason, there is a limitation to my data. A correction for water loss via the lung and skin in a half-hour period as well as faeces and urine disposal of the young might have improved the measurements, but the latter is difficult to determine accurately. Rehling and Trillmich (2007) excluded all observations in which urine secretion occurred after applying a similar methodology; however, this was not possible in my study since I only used a small sample size. By shortening the time with the mother, such a high amount of mass loss might be, in part, avoided and should be considered for further work. In support, Laurien-Kehnen and Trillmich (2003) demonstrated that the majority of milk transfer to the offspring is completed within 10 minutes after reunion with the mother.

Infant body weight growth remained comparable between male offspring born to gestationally stressed and undisturbed mothers throughout lactation, whereas early stressed female guinea pigs showed a non-significant tendency towards heightened weight gain. Also if corrected for litter size, stressed female offspring started with tendencially lower body weight at the beginning of lactation, but rapidly caught up weight and became heavier than controls (data not presented). These are interesting results for two reasons: firstly, it appears that stress-dependent reductions of maternal effort in terms of milk supply did not affect pup weight development and secondly, the female offspring's body condition was not supported by maternal lactation. Considering that guinea pig pups begin to feed on solid food on the first day of life and can meet their energy demands at least partly from sources other than maternal milk early in infancy (Künkele and Trillmich, 1997), pups may have compensated lowered milk transfer by increasing independent foraging (Laurien-Kehnen and Trillmich, 2004; Rehling and Trillmich, 2008). In a study by Kapoor and Matthews (2005), maternal strobe light exposure in late gestation significantly affected body growth over the post-weaning period (postnatal days 30-70) but not during lactation. In line with my results, it suggests that prenatal stress-induced effects on offspring weight development were not a response to maternal nutrient. Evidence derived from both animal and human clinical research support the programming of growth trajectories *in utero* (Epstein et al., 1977; Reinisch et al., 1978). For instance, Diego et al. (2006) found psychological distress in pregnancy significantly correlated to fetal growth development in women. Neuroendocrine responses to stress in the female and the resulting overexposure to maternal glucocorticoids and/or hormonal-induced changes in placental blood flow that affect the oxygen and nutrition delivery are suspected to function as developmental signal to the fetus (Donoghue et al., 2000). Greater glucocorticoid transfer occurs across the placenta of female compared with male mice fetus (Montano et al., 1993), which may account for more severe consequences in female offspring. Mueller and Bale (2006) assumed an interaction between the sex-specificity in the vulnerable to programming and the timing of maternal stress relative to birth. My work provides support for this idea as in the guinea pig, altered body growth in male but not female offspring is linked with experimental treatment during the last trimester of pregnancy (Emack et al., 2008; Kapoor and Matthews, 2008).

Faster-growing young females enter first estrus earlier (Trillmich et al., 2006). The up-regulation of body weight gain might thus be considered as an adaptive response, as in an environment of uncertainty and high risk of mortality the best strategy to increase fecundity is to reproduce early in life (Chisholm and Burbank, 2001).

Behavioural stress responsiveness in adults

Prenatal treated individuals were, although not significantly, less active during exposure to strobe light, indicated by a tendencially diminished expression of eating and locomotor activity. A similar trend was evident on measures of food consumption, proposing that offspring of stressed mothers showed decreased activity during the whole of stress exposure. These findings are in line with comparable assessments of longer latencies to move or eat (Thompson, 1957), reduced locomotion in an open-field (Poltyrev et al. 1996) as well as increased immobility time in forced swim tasks (Alonso et al., 2000; Welberg et al., 2000; Smith et al., 2004) in the progeny of females stressed in pregnancy. Based on reports by Caldji et al. (1998) and Smith et al. (2004) the heightened degree of emotionality or reduced coping ability is suggestive of exaggerated HPA axis reactivity. The potential mechanism for permanent modifications in the functional state of the HPA axis has been proposed to involve changes in the gene expression of glucocorticoid (GR) and mineralcorticoid receptors (MR) in selected brain regions, such as the hippocampus. The density of corticoidsteroid receptors is critical for feedback control of HPA activity as circulating glucocorticoids bind to GR and MR in the hippocampus to regulate corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) release. Hippocampal MR and GR levels are reduced in adult offspring born to gestationally stressed mothers, which diminishes the level of glucocorticoid negative feedback (Henry et al., 1994; Maccari et al., 1995; Barbazanges et al., 1996; Koehl et al., 1997). Centrally, the resulting up-regulated secretion of CRH is supposed to induce behaviour states such as fear and anxiety (Johnson et al., 1994).

In the present work, maternal effects on the offspring's behavioural phenotype were not substantial. Previously reported data indicate that the mechanisms underlying environmental programming depend on factors such as the severity and type of the stressor the pregnant animal was confronted with. The timing of maternal stress appears be of particular relevance with stronger influences during late gestation than during earlier stages of pregnancy (e.g. Koenig et al., 2005). While the exposure to strobe light is defined as moderate psychological stressor (Kapoor and Matthews, 2005), the most commonly used method on studies in rodents involves physical restraint of the pregnant animal, usually applied over several consecutive days, during the last trimester of gestation (Weinstock, 2008). Another option is that results vary based on different methodologies utilised to activate HPA function in the offspring (Matthews, 2002). The age of animals at time of testing is an important factor as well. Batuev et al. (1996) found lower amounts of locomotion under stress in one month old prenatally stressed rats; however, at the age of four months, these effects were no longer observable.

It is well established that prenatal stress programs enhanced stress responses in rat offspring (Weinstock, 2008). In the guinea pig, influences on the functional state of the HPA axis are less clear. Cadet et al. (1986) reported reduced basal and stress-induced cortisol release in the juvenile and adult progeny after maternal exposure to strobe light at a single day, gestational day 60 (gestational length=68 days). In contrast, an acute phase of maternal nutrient restriction on day 50 of gestation resulted in female offspring that exhibited elevated HPA axis responses to stressors (Lingas and Matthews, 2001), while male offspring of dams exposed to variable stressors over the second half of gestation were assessed to show decreased open-field activity (Emack et al., 2008). Moreover, male guinea pig offspring born to dams subjected to stress in late gestation have been reported to exhibit elevated plasma cortisol levels in response to strobe light exposure (Kapoor and Matthews, 2005). Effects therefore seem to be highly specific to the gestational period at which the pregnant mother was exposed to stress and have been hypothesized to depend on the timing of stress application in relation to the stage at which the fetal HPA becomes active (Weinstock, 2005). Maximal brain growth occurs around gestational day 50 in the guinea pig (Dobbing and Sands, 1970, 1979) and coincides with the first appearance of GR and MR in the fetal brain (Matthews, 1998). Matthews (1998) assumed that receptor systems and subsequent neuroendocrine function are most susceptible during this critical period of rapid development. Nonetheless, given my data, it was possible to demonstrate permanent effects on stress axis activity by maternal treatment during the early gestational period. Finally, my findings do not support sexually dimorphic outcomes in the sensitivity to neural programming (Weinstock et al., 1992, McCormick et al., 1995; Nishio et al., 2001) as tendencially increased fear reactivity was assessed regardless of sex.

Despite the fact that the prenatal environment had no dramatic effect on maternal behaviour in the current study, gestational stress appeared to induce a state of reduced maternal responsiveness towards the offspring. Strong social bonds between the mother and her young have been documented to positively influence offspring explorative behaviour with changes lasting over an individual's lifetime (Bowlby, 1969; Ainsworth et al., 1979). Disruptions in the affective mother-infant interaction by increased maternal rejection behaviour are likely to exert opposite effects on emotional reactions (Michel and Tyler, 2007). A direct link between environmental quality and maternal attachment, resulting in long-term behavioural consequences for the progeny, has been previously shown by Coplan et al. (1996, 1998) in the bonnet macaque. That is, maternal maintenance under high foraging conditions that required long periods of searching for food increased mother-infant conflicts. As adolescents, their offspring behaved more fearful and submissive than those of mothers reared under low foraging demand. Similarly, impairments in care-taking of pups following conditions to threat in pregnancy and, in turn, increased behavioural fearfulness in response to novelty have been assessed in juvenile and adult progeny of laboratory rats (Smith et al., 2004; Champagne and Meaney, 2006). According to Chisholm (1993) and Meaney (2001), stress-driven effects on the attachment process functions as a mechanism that provides the developing organism with a predictive signal of the quality of its future environment, adapting the resulting phenotype to the habitat condition experienced by the mother. Within an evolutionary context, the programming of sensitised HPA responses may serve to promote the detection of potential threat and avoidance learning with obvious fitness consequences in a hostile environment that requires enhanced vigilance (Cameron et al., 2005; Champagne and Meaney, 2006). O'Steen et al. (2002), for instance, demonstrated that under increased risk for predation, guppies that behaved more fearful in their escaping latencies showed higher survival.

Although cross-fostering experiments support the importance of postnatal determinants for offspring phenotypic development (Maccari et al., 1995), the experimental design used in my study does not allow for a differentiation between the role of pre- and postnatal maternal factors in modulation processes. Alternatively, stress-induced HPA responses in the pregnant mother are theorised to directly affect fetal CNS maturation via placental passage of maternal glucocorticoids (Sapolsky et al., 2000). As modifications of the fetal endocrine environment as well as behavioural maternal stimuli affect common neural targets, it is not possible to verify that perturbations in the mother-pup interaction would contribute independently to infant outcome. The observed variations in maternal care could be an epiphenomenon rather than a causative factor in the developmental plasticity of stress and fear responses as previously suggested by Macri and Würbel (2006).

Conclusion

In conclusion, I could demonstrate that early gestational stress exposure adversely affected some patterns of the behavioural mother-offspring relationship with most distinctive consequences for pup-directed aggressiveness. These results were consistent with impairments in the maternal lactational performance and may represent an adjustment of energy allocation in response to variable environmental conditions. Interestingly, diminished milk provision by the mother did not translate into profoundly altered pup body weight but might have been compensated by enhanced independent feeding of the young. Nonetheless, late-life effects on stress activational systems were evident, as subjects born to stressed mothers showed slightly exaggerated fear behaviour in stress challenging situations.

The extended period *in utero* and, as a result, the high state of maturation at birth and the somewhat loose attachment between mother and infant in the guinea pig, could be read to suggest that maternal environmental cues exert a more potent role during the prepartum developmental stage. However, the data derived from my experiments do not exclude the possibility that stress-induced changes in pup care and feeding might be important for determining the trajectories of environmental-dependent phenotypic variation and the fitness of offspring.

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Zusammenfassung

Umwelt- bzw. mütterliche Einflüsse in der perinatalen Entwicklungsphase können zu einer permanenten Veränderung („Programmierung“) des Phänotyps führen. Pränataler Stress ist ein in diesem Zusammenhang bekanntes Phänomen, welches insbesondere am Modellorganismus der Ratte experimentell belegt ist. Nachkommen von Müttern, welche in ihrer Schwangerschaft kurz- oder langfristigen Stresskonditionen ausgesetzt sind, weisen unter anderem Veränderungen in ihrem Verhaltensausdruck, so zum Beispiel in ihrer Stressreaktion oder Reproduktionsverhalten, auf. Ein möglicher Adaptationswert um die Fitness des Nachwuchses unter sich verändernden Umweltbedingungen zu steigern wird vermutet. Obwohl deren Folgen gut dokumentiert sind, ist der Mechanismus über welchen Umweltsignale den Phänotyp der Nachkommen beeinflussen weitgehend ungeklärt. So wird einerseits die mütterliche Sekretion des Hormons Kortisol, welches die Plazenta passieren kann, als Mediator diskutiert. Andererseits könnten auch Faktoren involviert sein, welche in die frühe postnatale Entwicklung eingreifen. Die wichtigste Komponente für Letzteres stellt die Versorgung durch die Mutter dar. Davon abgesehen, dass die mütterliche Laktation langfristige Konsequenzen für das Wachstum der Jungtiere mit sich trägt, ist das relative Ausmaß der sensorischen Stimulation durch die Mutter unter anderem für neurophysiologische Differenzierungsprozesse von Bedeutung.

Die Beeinflussung der mütterlichen Leistung durch Stresseinwirkung auf das schwangere Tier, sowie dessen Relevanz in der umweltinduzierten Entwicklungsprogrammierung der Nachkommen wurden im Rahmen meiner Diplomarbeit am Meerschweinchen (*Cavia aperea f. porcellus*) untersucht. Eine zwei bzw. einstündige (von 9-11 h sowie 16-17 h), in wöchentlichen Abständen erfolgende Bestrahlung mit Stroboskoplicht wurde als pränataler Stressor eingesetzt, beginnend in der Woche vor dem Eisprung sowie über die ersten beiden Drittel der Gestationsperiode (Tag -7, 0, 7, 14, 21, 28, 35 und 42 der Schwangerschaft; Tragzeit=~68 Tage). Verhaltensbeobachtungen zu Mutter-Jungtier Interaktionen fanden über die Laktationsperiode verteilt statt (Tag 1, 3, 6, 14 und 21 der Laktation) und darüber hinaus wurde die Milchproduktion der Weibchen durch Wiegen der Jungtiere vor und nach dem Säugen gemessen (Tag 4, 8 und 12 der Laktation). Die tägliche Gewichtszunahme der Jungtiere und die Verhaltensreaktionen der später adulten Tiere in einer Stresssituation wurden aufgezeichnet.

Es konnte festgestellt werden, dass gestresste Weibchen (n=6) im Vergleich mit Kontrolltieren (n=6) tendenziell häufiger ihre Jungen verließen und folglich weniger Zeit in Körperkontakt, einschließlich Säugen, mit diesen verbrachten. Die deutlichsten Diskrepanzen

zeigten sich in einem signifikant gesteigerten mütterlichen Aggressionsverhalten und einer verminderten Milchleistung. Dies könnte eine Strategie darstellen, um die Pflegezeit des Wurfes nach fordernden Bedingungen in der Schwangerschaft zu verkürzen. Weibliche Nachkommen gestresster Mütter wiesen gering abweichende Wachstumsraten, sowie beiderlei Geschlechter als Adulte eine tendenziell erhöhte Immobilität unter Stress auf, d.h. eine gesteigerte Stressempfindlichkeit. Obwohl der Einfluss pränataler Faktoren nicht ausgeschlossen werden kann und die Sensibilität gegenüber postnatalen mütterlichen Stimuli aufgrund der hohen Entwicklungsstands der Jungtiere beim Meerschweinchen vermutlich verringert ist, unterstützt meine Arbeit die Hypothese, wonach stressbedingte Beeinträchtigungen des mütterlichen Aufwands an den langfristigen Auswirkungen für deren Nachkommen beteiligt sein könnten.

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