Dexamethasone and hydrocortisone in neonatal rats

Tapering Dose of Hydrocortisone and Dexamethasone in Neonatal Rats: Effect on the Hypothalamic-Pituitary-Adrenal Response, Somatic and Neurological Development at Adolescence

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Dexamethasone and hydrocortisone in neonatal rats

Disclosures:

The authors have no financial relationships relevant to this article.

Grant support:

Supported by NIH grant RO1 HDDK37431
Dexamethasone and hydrocortisone in neonatal rats

Abstract
Exogenous administration of dexamethasone (DEX) or hydrocortisone (HC) to improve lung function and treat refractory hypotension is common in premature infants. This occurs at a time when the central nervous system is undergoing profound structural and functional transformations. This study was designed to evaluate the effect of tapering doses of DEX and HC in neonatal rats with timing of exposure corresponding developmentally to times these agents are used in clinical practice. A within litter design was used. Intramuscular DEX, HC or saline (VEH-control) were administered to newborn pups on postnatal days (PD) 5 and 6. Neurological development and growth was assessed weekly until pre-adolescence (PD33). At PD33 animals were submitted to novelty stress and anxiety testing using the light-dark preference box (L-D) and Corticosterone (CORT) and ACTH levels measured after novelty stress. Somatic growth was decreased in the DEX group compared with HC, and VEH animals at all ages. HC animals showed increased weight gain by PD33. Neurodevelopment was appropriate for age at all times measured. No treatment differences were appreciated between groups. CORT secretion decreased in DEX group compared with all other groups. HC animals had significantly higher ACTH levels long after all other groups returned to basal levels. Animals did not differ in L-D testing. A short tapering course of DEX and HC appears to be more benign than prolonged DEX exposure in early life. The effects of HC on Limbic-Hypothalamic-Pituitary-Adrenal (LHPA) axis, though subtle, raises concerns for altered LHPA function and potential risk for metabolic disorders in later life.

Key words: Neonatal rats, Neurodevelopment, Dexamethasone, Hydrocortisone, ACTH, Cortisol. HPA-axis

Precie:
The present study evaluated the effects of a short course of HC on somatic growth and the developing LHPA axis. A two day tapering dose glucocorticoid regimen was used.
Dexamethasone and hydrocortisone in neonatal rats

Introduction

Exogenously administered glucocorticoids, such as dexamethasone (DEX), can inhibit the stress response of the limbic-hypothalamic-pituitary–adrenal (LHPA) axis for prolonged periods and cause adverse effects on the growth and development in both animals and humans (1-7). In humans, DEX has been used in tapering courses lasting 28 days or more in the extremely low birth weight (ELBW) infants to reduce the morbidity of chronic lung disease (CLD) (8-11). In practice, the timing of DEX use in ELBW infants has corresponded to corrected gestational ages when the central nervous system (CNS) is undergoing profound structural and functional transformations, making it vulnerable to external influences such as DEX exposure. Follow up studies in the late 1990’s provided evidence of significant increase in neurodevelopmental dysfunction in preterm neonates who received prolonged courses of DEX to reduce morbidity associated with chronic lung disease (12). As a result of such findings DEX use has been significantly reduced in clinical practice and when used the courses are substantially shorter (13-15). While the use of DEX for chronic lung disease has been restricted substantially, few alternative treatment options are available.

In recent years, hydrocortisone (HC) has emerged as a treatment option for premature infants with respiratory distress, chronic lung disease (CLD), and hypotension (16,17,18,19). Hypotension can be secondary to multiple causes (e.g. PDA, sepsis). In practice, when used for CLD in preterm infants, DEX and HC are used later and later in chronological age (20, 17, 21,18,19, 22). The use of low-dose HC increases the likelihood of survival in premature infants without chronic lung disease (23,24). As with early use of DEX, the long term effects of HC on the developing CNS in preterm and term neonates remains unknown, though small studies suggest that HC may not significantly predict adverse neurodevelopment (25, 17,18,19).

In recent years, animal models have been developed in an attempt to better understand the neurological consequences of perinatal glucocorticoid exposure and long term neurodevelopmental outcomes (26,27,28). When interpreting behavioral and physiologic effects of neonatal glucocorticoids in any developmental model, it is assumed that although timing differs significantly between species, the general sequence of brain growth is similar. An appropriate rodent model that uses these relationships has been developed in our laboratory. Most important in our decision to use a rodent model is the ability to take advantage of the unique neurodevelopmental profile present...
Dexamethasone and hydrocortisone in neonatal rats
during the neonatal period. It is estimated that on postnatal day 10 the rodent brain is roughly equivalent to that of the human brain of 38 to 40 weeks post-conception(29,30). Extrapolating from this, the brain of a rodent pup at birth (postnatal day 1-PD1) corresponds to that of a human fetal brain at or near 21-22 weeks gestation (30, 31,32). The brain of a PD3 pup approximates that of a 24-26 wk human and PD6 approximates a 30-32 wk human. We therefore modeled the timing of DEX exposure in this model to that utilized in premature infants. We have demonstrated changes in LHPA function and anxiogenic behavior in adolescent animals exposed to DEX during the first week of life when DEX was given at PD3, 4, 5 and 6, indicating long term repercussions of such treatment (6,33).

In view of present neonatal practices using short course DEX or HC in high risk premature infants, the purpose of the present study was to evaluate the effects of a short course of HC on somatic growth and the developing LHPA axis. We chose a two day tapering dose regimen starting on PD5 to capture a common clinical scenario in the human infant where glucocorticoid exposure is given later in life to minimize chronic lung morbidity. As in our previous DEX neonatal rat model, we started with a HC dose equivalent to that used in the human neonate, comparing it to DEX as well as untreated controls.

**Materials and Methods**

**Animals**

Litter management and animal handling of neonatal rats in this study was similar to that reported from our laboratories previously (6, 34) Adult Sprague-Dawley rats (Charles Rivers, Wilmington, MA) were housed and treated according to Guide for the Care and Use of Laboratory animals. All animals were kept under constant temperature (25 ± 2°C) and photoperiodicity (14:10h light-dark cycle) and provided with food and water ad libitum. Animals were mated using one to one mating system (1F:1M). Assuming a 21-day gestation, pregnant females were housed separately, starting on day 18. They were then checked twice daily until pups were born. The day of the birth of the pups was designated as postnatal day one (PD1).

Consistent with our previous studies, each litter was sexed and culled to 12 pups (6M: 6F) on the second day of life. Pups were randomly selected as male: female pairs from 3 different dams giving birth the same day. This ensured both genetic diversity, equality in nutrition and maternal care within litters. Within each litter, pups were randomly
Dexamethasone and hydrocortisone in neonatal rats

chosen to one of three groups: VEH, DEX, or HC. Pups were permanently marked for identification according to their treatment group by clipping the outer toe tip of the rear left paw (DEX), rear right paw (HC) and front left paw (VEH). These permanent markings were visible throughout the experiment period negating the need for further animal tagging and minimized handling for identification. A total of 144 animals were studied.

Drug Treatment

Pups were removed from their mothers between the hours of 1100 and 1300. Animals in the DEX group received an intramuscular (IM) injection of DEX in a tapering dose of 0.5 mg/kg on PD5 and 0.1 mg/kg on PD6. HC animals received an IM HC injection at 5.0 mg/kg on PD5 and 1.0 mg/kg on PD6. VEH animals received an equivalent volume of sterile normal saline IM. The study protocol and days of treatment are depicted in Table 1.

Somatic Growth: Lengths and weights were measured for each pup in each treatment group before handling or treatments on PD5 and PD6. Animals were also measured and weighed on PD 7, 14, 20 and prior to stress testing on PD33. At each age, length was measured from the nose to the base of the tail (head-rump length).

Neurological assessment: Procedures for the neurological assessment were performed on PD 7, 14 and 20 and were adapted from work of several investigators (26, 27, 28). Results using this method of neurodevelopmental assessment have also been reported by our group (6, 34) Each pup underwent testing at the same time on each designated neurodevelopmental assessment day. Two trained assistants measured neurodevelopmental indices in all animals using a rating of 0-5 with a score of 5 corresponding to maturity. Neurodevelopment assessment in the neonate included posture, righting reflex, postural flexion and extension, vibrissa placing, fore limb and hind limb placing, geotaxis, and bar hold and cross extension. Physical maturity was measured by observing eye opening, ear opening, ear folding, ear twitch, fur development and tooth eruption. In order to obtain meaningful analysis of physical and neurological assessment these observations were grouped, as presented in Table 2. The grouping was done as follows: physical maturation (posture, eye opening, ear opening and fur development); teeth eruption (maxillary and mandibular eruption); reflexes (ear twitch, righting reflex, postural flexion and extension and fore and hind limb
Dexamethasone and hydrocortisone in neonatal rats

grasp reflex); sensory complex (Vibrissa placing and geotaxis); and gross motor activity (fore and hind limb placing, cross limb and bar hold).

**Brain Weight:** On PD8, one pup per treatment group was sacrificed to obtain brain weights. Brain weights were also obtained during necropsy on PD33.

**Behavioral Testing:** A total of 144 pups were ultimately studied at adolescence.

The light-dark preference box was used to evaluate locomotion and investigatory behavior of the animals on PD33 in a manner previously reported by this laboratory (34). Preference for darkness and decreased activity are gross measurements of anxiety. At age PD30, animals were acclimated to handling before the start of the procedure. Handling consisted in transporting the cage to the test room and removal from the cage. This was done for three consecutive days. The testing apparatus was a covered 30 x 60 x 30 cm Plexiglas shuttle-box with a computerized monitor, two equal sized compartments (light and dark) with a 12-cm wide opening and a stainless steel grid floor suspended above corncob bedding. The light compartment was constructed of white Plexiglas and brightly illuminated. The dark compartment was constructed of black Plexiglas and minimally illuminated. Fluorescent lights above the box provided the illumination. To begin the session, the animal was placed in the dark compartment and the timer set to start. Each animal’s locomotor activity, the time spent in each compartment, the number of transitions and the latency to leave the dark were scored. Locomotor activity as well as time spent in each compartment was monitored by photocells located on the wall of each box, with the number of photocell beams interrupted per unit time recorded by microprocessor. The number of transitions were recorded manually. Total testing time was 5 minutes (6).

**Adrenocortical Response to Novelty Stress:** After light-dark preference testing was completed, blood was collected from the animal’s tail vein by cannulation at 15, 30 and 60 min after the beginning of the testing session. The last blood sample was collected 90 min after the start of the test, at which time the animals were decapitated. A pre-stress blood sample was obtained the day prior to the light-dark box as the animal was acclimated to handling. The time of the pre-stress sample corresponded to the same time of the start of the procedure on the following day. Blood samples were collected in pre-chilled tubes containing EDTA, placed on ice and subsequently spun at 2000 rpm for 7
Dexamethasone and hydrocortisone in neonatal rats

min. The plasma was separated and stored at -20°C until assayed for corticosterone (CORT) and adrenocorticotropic (ACTH) hormone concentrations.

**Hormonal Assays**

Corticosterone levels were measured using a commercially available corticosterone $^{125}$I radioimmunoassay kit (Cat. #07-120102, MP Biomedicals LLC, Diagnostic Division, Orangeburg, New York) after plasma samples were diluted to 1:200 in Phosphosaline gelatin buffer (pH 7.0). The intra- and inter assay CVs for corticosterone were 4.4% and 6.5%, respectively.

Plasma adrenocorticotropic hormone concentrations were measured using the ImmuChem™ Double antibody hACTH $^{125}$I RIA kit (MP Biomedicals LLC. Orangeburg, NY 10962). This assay uses un-extracted plasma with EDTA as an anticoagulant. A standard curve was generated using specified amount of antibody reacted with radiolabeled hormone per manufacturer’s instructions. This standard curve was then used to measure unknown amounts of ACTH in the study samples. The intra and inter day coefficient of variation for the assay was 6.8% and 10.7%, respectively.

**Statistical Analyses**

Body weight and length were analyzed using repeated measures ANOVA. Total and individual neurological scores were averaged across groups and analyzed using nonparametric test (Kruskal-Wallis). The effect of sex and HC or DEX treatment on various neurodevelopmental outcomes on PD 7, 14 and 21 was compared. Brain weights, light-dark behavior and plasma hormonal levels (ACTH and CORT) were analyzed using repeated measures ANOVA. Significance was indicated by a p value p<0.05. Once significance was observed by ANOVA, the Fisher’s least significant difference (Fisher’s PLSD) method was utilized for further pair-wise comparisons.

**Results**

8
**Dexamethasone and hydrocortisone in neonatal rats**

**Somatic Growth**

The statistically significant relationships for the parameters measured under somatic growth are presented in Table 3 and figures 1 and 2.

**Weight:** The overall change in weight amongst the three treatment groups on days PD5-PD20 is presented in Figure 1-Panel A. As shown, weight gain in the DEX treated animals was significantly lower than in the HC and VEH groups on days PD6 - PD20 (P<0.05). There were no sex differences during this period. The interaction between sex and treatment was significant for PD33. Further analysis did not result in any difference in the male animals in the three treatment groups (Panel B) but DEX treated female animals were significantly smaller than the VEH group on PD33 (Panel C; P<0.05).

**Length:** The results of effect of the three treatments on length are presented in Figure 2-Panel A and B. Initial examination revealed a significant interaction between sex by time (P<0.0002) and treatment by time (P< 0.0018). Thus, males and females were analyzed separately. DEX treated female and male animals were significantly shorter compared with HC and VEH on PD 6, PD7, PD14 and PD20 (P<0.0001). On PD33 males in all 3 treatment groups had similar length. However, DEX treated females were significantly shorter than those in the VEH or HC group on PD33 (P< 0.001).

**Neurological Development**

Figure 3 -Panels A-E depict the results of the neurological development. Animals were studied on three different postnatal time points: PD 7, PD14 and PD21. Following repeated measure ANOVA, we did not observe significant effect of sex on any neurodevelopment exams within treatment groups. Male and female data was therefore collapsed for further analysis. There were neurological functioning differences across time with a significant age by neurological assessment interaction (p < 0.0001). The post hoc analysis indicated that the pace of development was not significantly different for the DEX group compared with VEH or HC for all neurological measurements across the time of assessment with the exception of physical maturation on PD7 and teeth eruption on PD21. The teeth eruption score was significantly lower for DEX animals compared with HC and VEH on PD21 (Panel A; P<0.05)
Dexamethasone and hydrocortisone in neonatal rats

and the physical maturation score was significantly lower for DEX compared with VEH on PD7 (Panel D; P<0.05). By day 21 the physical maturation was similar in all three treatment groups.

Behavioral Assessment

On testing for place-preference in the light-dark box, there was no difference between treatment groups or between males and females on latency to enter the light box when placed in the dark compartment (p>0.05). Similarly, the locomotor activity and time spent in dark or light compartment was similar for males or females across groups. Thus, all Light-Dark Box Behavioral measures obtained were not significantly different between treatment groups irrespective of sex (data not shown).

Brain Weights

On PD33 the brain weights of males and females were significantly different within the DEX and HC treatment groups (ANOVA, p<0.0001). The post hoc analysis showed that DEX treated animals were different from those treated with HC. Males had greater brain weights when compared to females in both groups. However, when brain weights were normalized for body weight the ratio of brain:body weight was not significantly different between treatment groups or for males vs females within each treatment group (data not shown).

Pituitary Response to Novelty Stress

Results of novelty stress are presented in Figure 4-Panels A to D. Repeated measures ANOVA of ACTH response to novelty stress revealed no differences between males and females, therefore the data were collapsed across this variable. We observed significant differences among the groups with HC treated animals having greater mean ACTH levels when compared to DEX animals (p=0.04). Analysis of the individual profiles of ACTH release at each time point showed a significant difference across groups at 30 and 90 min (Figure 4, Panel A). At 30 min, HC treated animals had elevated ACTH levels when compared to DEX and VEH animals. HC treated animals also had significantly higher ACTH levels at 90 min, a time that all other groups had returned close to basal ACTH levels. Consistent with this profile, the AUC analysis showed significantly greater overall ACTH secretion by HC treated animals (Figure 4 –Panel C).

Adrenocortical Response to Novelty Stress

10
Dexamethasone and hydrocortisone in neonatal rats

Repeated measures ANOVA revealed that the adrenocortical activation by novelty stress was similar in both male and females, therefore the analysis was collapsed across this variable and revealed significance across groups (p=0.02). A significant CORT by group interaction was also present (p=0.002). The DEX treated animals had a lower response when compared to all other groups. When we analyzed the group profiles using factorial measure ANOVA, all groups showed peak CORT levels at 15 min, with the VEH group having the greatest response (p<0.05) All groups returned to baseline CORT levels by 90 minutes post-stress. However, the HC and VEH treated animals remained with significantly higher CORT levels at 90 min when compared to DEX groups. Animals treated with DEX had significantly lower CORT levels much earlier than the other groups, at 30 min post stress (p<0.05)(Figure 4–Panel B). These data were consistent with the AUC analysis which revealed that the overall secretion of CORT in response to novelty stress was decreased for the DEX treated animals when compared to all other groups (p<0.001; Figure 4-panel D).

Discussion

Long term DEX therapy in the newborn period has been shown to lead to undesirable neurological sequela in extremely low birth weight infants (35, 36). Consequently, the use of DEX in current clinical practice has decreased markedly over the past decade. Moreover, the comparative safety of other therapeutic alternative, such as HC or short course DEX, has been minimally explored (16, 38, 23, 24).

The present study has used an animal model to ascertain early and long term effects of DEX and HC, at doses commonly used in clinical practice, given during first week of life. The timing of drug administration was important in our study because this is a period of time that captures a common clinical scenario in the older extremely premature infant that is developing severe chronic lung disease. It is also a time when the neonatal brain appears to be most vulnerable to insults (29,30).. It is important to note that this rat model in no way is attempting to mimic the shorter 48-h glucocorticoid bursts used for refractory hypotension or prolonged postnatal treatment with other glucocorticoids. Keeping in mind these differences, our findings suggest that a short course of DEX or HC during the neonatal period may still have long-lasting consequences, though mild in general.

Somatic Growth

11
Dexamethasone and hydrocortisone in neonatal rats

Animals given DEX on PD5 and PD6 had a slower rate of weight and length gain when compared to HC treated animals and control groups (VEH) resulting in a smaller animal in the DEX group for both weight and length during the entire study period, up to pre-adolescence (PD33). As in our previous DEX study, we presume that observed differences in somatic weight are due to a direct effect of DEX on catabolism and tissue accretion (31). It has been shown that DEX prevents adequate growth, rapidly inducing protein catabolism beyond the capacity for an anabolic state and resulting in reduced growth and lean body mass (37,38, 39,40). Alternatively, inadequate nutritional intake during the postnatal period due to inability to attach to the mother’s nipples or poor suckling is a possibility we cannot exclude. It has been shown that the dam spends more time providing nutrition, stimulation, and warmth to a litter that is perceived to have poor health (41-44). Therefore, we favor the explanation that DEX at the given doses altered the anabolic environment that is known to be present in the stress hyporesponsive period in the rat (4,45).

The effect on the handled pups is not surprising, since ‘touch’ during the neonatal period has been shown to be critical for normal growth and development both in animal models (46,47) and in humans (48). Increased somatic growth at pre-adolescence observed in those animals that were treated with HC early in life is difficult to explain. It is possible that the potency of HC at the given doses was significantly less than that of DEX. If so, it is possible that the known negative effects of glucocorticoids on bone growth plates was not fully achieved (49). However, this does not explain the enhanced growth. A possible explanation is an effect of HC at the given doses given early in life on insulin action. Glucocorticoid levels achieved by the early treatment could induce an insulin resistant state that promotes adiposity (50). Elevated insulin can act as a growth factor because of its structural similarity to insulin-like-growth factors (51,52). The important distinction here is that the detrimental effects of the more potent DEX treatment given early in life is not observed in animals treated with the less potent glucocorticoid during the same developmental period. While this may indicate that HC is a ‘better’ glucocorticoid agent in early life, there are other concerns such as the catch-up growth observed in our HC treated animals which favored visceral fat deposition which could increase the risk for insulin resistance and subsequent diabetes later in life by promoting obesity in adulthood (53,54). This will probably be addressed in future studies.
Dexamethasone and hydrocortisone in neonatal rats

Neurodevelopment and physical maturation

We did not observe any gross neurological deficits in the animals that received glucocorticoid treatment early in life. Primitive reflexes appeared and disappeared in a defined sequence during development, with an absence or persistence of any reflex beyond the expected time period indicating significant brain dysfunction (55). Since DEX or HC did not have any significant effect on these reflexes nor had only a transient effect, it appears that these treatments do not have any long term neurological sequelae. Clinical animal data suggest a defect in myelination in neonatal pups treated with long term DEX (55-59). We did not perform neuroanatomical studies to determination the extent of myelination in these pups. Evidently, our functional observations do not support this effect. However, it remains unclear at present whether permanent CNS changes have occurred that are too subtle to detect by our assessment.

DEX-treated animals also had decreased gross brain weights. However, when corrected for body weight, brain weights did not differ between groups or sex of the animal. Similar findings have been reported in human clinical studies where a 30% reduction in cerebral tissue volume is observed in premature infants treated with DEX compared with untreated age-matched controls(56). Therefore, even though DEX effect can be corrected by body weight, the brain is smaller and it is possible that DEX affected neurogenesis, gliogenesis, or myelination within specific vulnerable brain structures.

Stress Reactivity to a Novel Environment

When exposed to the novel environment of the place preference box at pre-adolescence, the glucocorticoid treated animals did not display anxiety like behaviors. It is possible that the age we tested may be too early to see behavioral effects. However, in a previous study, we found that animals treated with DEX from postnatal day 3 to 6 were less active in the light-dark environment of the place preference box on PD33(6) The place preference box is a less threatening environment when compared to the elevated plus maze (EPM) or open field tests (OF) (33). More rigorous testing may have uncovered behavioral disturbances not evident with the less stressful place preference testing.
Dexamethasone and hydrocortisone in neonatal rats

We found that both HC and DEX treated animals had an altered adrenocortical response to novelty stress. DEX treated animals had an adequate corticosterone response but low when compared to control and HC treated animals. Despite this low corticosterone response, DEX animals successfully terminated their ACTH response. Similarly, ACTH inhibition was present in VEH treated group.

In contrast, the ACTH response to novelty was significantly greater in the HC treated animals when compared to all other groups. This indicates slow termination to baseline. Therefore, treatment with HC did not alter the adrenal response to stress but lead to enhanced neuroendocrine response evidenced by increased ACTH levels. This suggests the possibility of altered LHPA related brain circuitry resulting in a faulty feedback mechanism as a consequence of this early life treatment. Previously, we have reported decreased rat glucocorticoid receptor mRNA expression in the hippocampus but no change in mineralocorticoid activity in adult rats that were exposed to DEX from PD3 to PD6 (60). This combination of corticoid receptor expression is linked to faulty feedback and altered behavior (61).

Glucocorticoid and mineralocorticoid receptor expression has been shown to develop primarily in the postnatal period in neonatal rat brains (4). This developmental phase corresponds to a very critical neurodevelopmental period in the human premature infant (29, 62). It is during this equivalent period that the brains of premature infants are exposed to these exogenous steroids (63,64). Therefore, adverse effects on the brain remain a serious concern when using corticosteroids in the postnatal period.

In conclusion, exposure to a short course of DEX or HC that mimics clinical practice in neonatal intensive care units has long-lasting effects on somatic growth and neuroendocrine function in the rat. One could interpret that the HC treatment is benign when compared to DEX exposure in the premature infant. The effects of HC, though subtle, raise concerns about altered LHPA function and metabolic alterations that may place the infant at risk for future metabolic disorders depending on the postnatal environment (65,66, 67,68, 69). Premature infants treated with HC early in life may be vulnerable to maladaptive endocrine and behavioral strategies that may not be recognizable until later in life. Detailed clinical research is needed to clarify these possibilities that may have important implications on learning, mood and ultimately quality of life in long-term survivors of prematurity.
Dexamethasone and hydrocortisone in neonatal rats

Table 1: Schematic of the Procedures and Age of the animals when these were performed

<table>
<thead>
<tr>
<th>Postnatal Day</th>
<th>PD 2</th>
<th>PD5</th>
<th>PD6</th>
<th>PD7</th>
<th>PD8</th>
<th>PD14</th>
<th>PD20</th>
<th>PD33</th>
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<tbody>
<tr>
<td>Procedures</td>
<td>Culled</td>
<td>Body</td>
<td>Body</td>
<td>Body</td>
<td>Brain</td>
<td>Body</td>
<td>Body</td>
<td>Brain Weight</td>
</tr>
<tr>
<td>Drug Regimen</td>
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<td>Weight</td>
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<td>Weight</td>
<td>Weight</td>
<td>Weight</td>
<td>Light-Dark Preference</td>
</tr>
<tr>
<td>A*</td>
<td>DEX 0.5mg/kg</td>
<td>DEX 0.1mg/kg</td>
<td>Neuro</td>
<td>Neuro</td>
<td>Neuro</td>
<td>Light-Dark Preference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tests</td>
<td>DEX 0.5mg/kg</td>
<td>DEX 0.1mg/kg</td>
<td>Neuro</td>
<td>Neuro</td>
<td>Neuro</td>
<td>Light-Dark Preference</td>
<td></td>
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</tr>
<tr>
<td>Procedures</td>
<td>Culled</td>
<td>Body</td>
<td>Body</td>
<td>Body</td>
<td>Brain</td>
<td>Body</td>
<td>Body</td>
<td>Brain Weight</td>
</tr>
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<td>Weight</td>
<td>Weight</td>
<td>Weight</td>
<td>Weight</td>
<td>Weight</td>
<td>Light-Dark Preference</td>
</tr>
<tr>
<td>B*</td>
<td>HC 5mg/kg</td>
<td>HC 1mg/kg</td>
<td>Neuro</td>
<td>Neuro</td>
<td>Neuro</td>
<td>Light-Dark Preference</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Animals receiving drug treatment were matched within litter with animals that were treated with vehicle injections.
Dexamethasone and hydrocortisone in neonatal rats

Table 2. Neurological Examination Rating Scale: Physical and Neurodevelopmental Assessment

<table>
<thead>
<tr>
<th>Physical Assessment</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
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<td>Full flexion</td>
<td>Partial flexion</td>
<td>Full extension</td>
<td>Partial extension</td>
<td>Normal</td>
<td></td>
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<tr>
<td>Eye opening</td>
<td>Closed</td>
<td>Occasional opening</td>
<td>Occasional opening</td>
<td>Fully open</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ear opening</td>
<td>Closed</td>
<td>Occasional opening</td>
<td>Occasional opening</td>
<td>Fully open</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ear folding</td>
<td>Barely visible</td>
<td>Completely folded</td>
<td>Fine hairs</td>
<td>Partially folded</td>
<td>Fully open</td>
<td>Fully open</td>
</tr>
<tr>
<td>Fur development</td>
<td>No fur</td>
<td>Fine hairs</td>
<td>Partial fur</td>
<td>Mostly fur</td>
<td>Full fur</td>
<td></td>
</tr>
<tr>
<td>Maxillary eruption</td>
<td>No teeth</td>
<td>Initial maxillary</td>
<td>Partial maxillary</td>
<td>Partial maxillary</td>
<td>Mostly full</td>
<td>Maxillary full</td>
</tr>
<tr>
<td>Mandibular eruption</td>
<td>No teeth</td>
<td>Initial mandibular</td>
<td>Partial mandibular</td>
<td>Partial mandibular</td>
<td>Mostly full</td>
<td>Mandibular full</td>
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<td>Neurodevelopment Assessment</td>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
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<td>Ear twitch response</td>
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<td>Weak response</td>
<td>Moderate response</td>
<td>Strong response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Righting reflex</td>
<td>On side full flexion</td>
<td>Partial flexion</td>
<td>Extension predominates</td>
<td>Partial extension</td>
<td>Normal position</td>
<td></td>
</tr>
<tr>
<td>Grasp reflex</td>
<td>No grasp</td>
<td>Weak grasp</td>
<td>Moderate</td>
<td>Full grasp</td>
<td></td>
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</tr>
</tbody>
</table>
### Sensory Complex

<table>
<thead>
<tr>
<th>Forelimb</th>
<th>Grasp reflex hind limb</th>
<th>Grasp</th>
<th>Moderate grasp</th>
<th>Full grasp</th>
</tr>
</thead>
<tbody>
<tr>
<td>No grasp</td>
<td>Weak</td>
<td>Moderate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross extensor</td>
<td>Absent</td>
<td>Weak</td>
<td>Moderate</td>
<td>Strong</td>
</tr>
<tr>
<td>No response</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vibrissa placing</td>
<td>No response</td>
<td>Weak response</td>
<td>Moderate response</td>
<td>Strong response</td>
</tr>
<tr>
<td>Forelimb placing</td>
<td>No response</td>
<td>Weak response</td>
<td>Moderate response</td>
<td>Strong response</td>
</tr>
<tr>
<td>Hind limb placing</td>
<td>No response</td>
<td>Weak response</td>
<td>Moderate response</td>
<td>Strong response</td>
</tr>
<tr>
<td>Geotaxis</td>
<td>No response</td>
<td>Pivoting predominates</td>
<td>Turns 180°</td>
<td>Strong response</td>
</tr>
</tbody>
</table>

### Gross Motor

<table>
<thead>
<tr>
<th>Forelimb</th>
<th>Hind limb placing</th>
<th>Bar hold</th>
<th>Postural extension</th>
<th>Postural flexion</th>
</tr>
</thead>
<tbody>
<tr>
<td>No response</td>
<td>No response</td>
<td>No grasp</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Weak response</td>
<td></td>
<td>Weak grasp</td>
<td>Partial</td>
<td>Partial</td>
</tr>
<tr>
<td>Moderate response</td>
<td></td>
<td>Moderate grasp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong response</td>
<td></td>
<td>Full grasp</td>
<td>Present</td>
<td>Present</td>
</tr>
</tbody>
</table>

A score of 0 represents complete immature physical development or immature response on exam. A score of 5 represents completely mature physical maturation or response on exam.
Dexamethasone and hydrocortisone in neonatal rats

Table 3: Mean Weight and Length increase over time by treatment group

<table>
<thead>
<tr>
<th></th>
<th>WEIGHT (GMS)</th>
<th>LENGTH (MM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEAN</td>
<td>SD</td>
</tr>
<tr>
<td>VEH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD5</td>
<td>11.09</td>
<td>0.98</td>
</tr>
<tr>
<td>PD6</td>
<td>12.59</td>
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<td>PD14</td>
<td>32.97</td>
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<td>PD20</td>
<td>47.37</td>
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<td>PD33</td>
<td>118.30</td>
<td>10.58</td>
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<tr>
<td>HC</td>
<td></td>
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</tr>
<tr>
<td>PD5</td>
<td>10.97</td>
<td>0.98</td>
</tr>
<tr>
<td>PD6</td>
<td>12.11</td>
<td>1.09</td>
</tr>
<tr>
<td>PD7</td>
<td>14.03</td>
<td>1.17</td>
</tr>
<tr>
<td>PD14</td>
<td>32.97</td>
<td>2.40</td>
</tr>
<tr>
<td>PD20</td>
<td>47.37</td>
<td>3.31</td>
</tr>
<tr>
<td>PD33</td>
<td>117.11</td>
<td>11.96</td>
</tr>
<tr>
<td>DEX</td>
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<tr>
<td>PD5</td>
<td>10.86</td>
<td>1.13</td>
</tr>
<tr>
<td>PD6</td>
<td>10.40</td>
<td>1.02</td>
</tr>
<tr>
<td>PD7</td>
<td>11.23</td>
<td>1.13</td>
</tr>
<tr>
<td>PD14</td>
<td>28.52</td>
<td>1.93</td>
</tr>
<tr>
<td>PD20</td>
<td>42.42</td>
<td>2.38</td>
</tr>
<tr>
<td>PD33</td>
<td>109.59</td>
<td>9.48</td>
</tr>
</tbody>
</table>

PD Postnatal day; VEH Vehicle; DEX-dexamethasone; HC Hydrocortisone
Dexamethasone and hydrocortisone in neonatal rats

Figure 1: Weights obtained from PD5 to PD 20 (panel A) and PD 33 (panels B and C) in the males vs females in the three treatment groups. Weight gain in the DEX treated animals was significantly lower than in the HC and VEH groups on PD6-PD20 (P<0.05). There were no sex differences during this period. (Panel A). DEX treated male animals were not significantly different from VEH or HC on PD33 (Panel B). DEX treated female animals were significantly smaller compared with VEH (P<0.05) but not to HC (Panel C).
Figure 1: Weight measurement separated by treatment groups from PD5 to PD 20 (panel A) and PD 33 (panels B and C) in the males vs females in the three treatment groups. Weight gain in the DEX treated animals was significantly lower than in the HC and VEH groups on PD6-PD20 (P<0.05). There were no sex differences during this period (Panel A). DEX treated male animals were not significantly different from VEH or HC on PD33 (Panel B). DEX treated female animals were significantly smaller compared with VEH (P<0.05) but not to HC (Panel C).
Dexamethasone and hydrocortisone in neonatal rats

Figure 2: Length measurements in animals separated by treatment and sex. from PD5 to 33 in the males vs females in the three treatment groups. males vs females in the three treatment groups. DEX treated female animals were significantly shorter in length compared with HC and VEH on PD6- PD33(P<0.0001) (Panel A). DEX treated male animals were significantly shorter than VEH and HC on PD6-PD20 (P< 0.001) but on PD33 there was no difference in length among the three treatment groups.(Panel B)
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Dexamethasone and hydrocortisone in neonatal rats

Figure 3: Comparison of neurobehavioral outcomes (Physical maturation) between treatment groups on day PD7, PD14 and PD21. There was no significant effect of sex or treatment on any neurodevelopment exams within treatment groups (p< 0.0001) with the exception of physical maturation (Panel D) and reflexes (Panel E); these two domains had limited change across the times of assessments.
Dexamethasone and hydrocortisone in neonatal rats

Figure 3: Comparison of neurobehavioral outcomes (Physical maturation) between treatment groups on day PD7, PD14 and PD21. There was no significant effect of sex or treatment on any neurodevelopment exams within treatment groups (p < 0.0001) with the exception of physical maturation (Panel D) and reflexes (Panel E); these two domains had limited change across the times of assessments.
Figure 4. Pituitary and Adrenal Response to Novelty Stress at Post-natal Day 33. HC treated animals had a greater mean ACTH levels (pg/mL) and AUC than VEH and DEX at 30 and 90 minutes post-stress suggesting greater overall pituitary secretion (Panels A and C). DEX treated group had significantly lower CORT levels at 30 and 90 min than VEH and HC groups (Panels B and D), indicating best termination of the stress response when compared to other groups. *: p<0.05, vs VEH and HC; #: p<0.05, vs VEH; †: p < 0.05, vs DEX
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