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# Effects of tapering neonatal dexamethasone on rat growth, neurodevelopment, and stress response

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STANLEY J. WATSON, JR.,<sup>2,3</sup> AND CHARLES R. NEAL, JR.<sup>1,2</sup>  
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**Flagel, Shelly B., Delia M. Vázquez, Stanley J. Watson, Jr., and Charles R. Neal, Jr.** Effects of tapering neonatal dexamethasone on rat growth, neurodevelopment, and stress response. *Am J Physiol Regulatory Integrative Comp Physiol* 282: R55–R63, 2002.—Dexamethasone is commonly used to lessen the morbidity of chronic lung disease in premature infants, but little is known regarding neurological consequences of its prolonged use. To study neurological effects of dexamethasone, we have developed a rat model in which newborn pups are exposed to tapering doses of dexamethasone at a time corresponding neurodevelopmentally to human exposure in the neonatal intensive care unit. On postnatal *day* (PD) 2, litters were divided into three groups: 1) handled controls, 2) saline-injected animals, and 3) animals injected with tapering doses of intramuscular dexamethasone between PD 3 and 6. Somatic growth and brain weight were decreased in dexamethasone-treated animals. Dexamethasone-treated animals demonstrated delays in gross neurological development on PD 7 and 14 but not PD 20. In late adolescence (PD 33), dexamethasone-treated animals were less active in light and dark environments, while demonstrating a blunted serum corticosterone response to a novel stress. The dissociation between behavioral and hormonal stress responsiveness suggests that neonatal dexamethasone exposure permanently alters central nervous system function, particularly within the neuroendocrine stress axis. This may lead to increased risk for learning impairment and maladaptive responses to the environment.

steroids; prematurity; brain; corticosterone; limbic-hypothalamic-pituitary-adrenal axis

IN EXTREMELY LOW BIRTH WEIGHT (ELBW) infants with severe respiratory distress syndrome, postnatal dexamethasone (Dex) is often used to lessen the morbidity of chronic lung disease. Even though clinical trials fail to consistently demonstrate significant improvement in mortality or length of stay, prolonged courses of up to 42 days are still used in many centers (3, 13, 25, 26). The time of treatment for premature infants corresponds to the viable perinatal period from 24 to 40 wk postconception, when the human central nervous system (CNS) undergoes profound structural and functional transformations, making it particularly vulnerable to a variety of external influences. Common side

effects of Dex use in ELBW infants include hypertension, bowel perforation, infection, ventricular hypertrophy, catabolic changes, and alteration of the limbic-hypothalamic-pituitary-adrenal (LHPA) axis (1, 7, 11, 19, 27, 33, 34). Despite these observations, very little data have been generated with regard to possible glucocorticoid effects on the developing CNS.

Clinical studies have demonstrated that premature infants receiving long-term Dex therapy have reduced linear growth, decreased weight gain, and smaller head circumferences (8, 21, 42). During the acute phase of Dex exposure, changes in gross neuromotor function have also been noted (9, 56). Not surprisingly, recent clinical studies have linked Dex therapy to long-term neurological effects, including an increased incidence of cerebral palsy at 1 yr corrected for age and decreased cerebral volume as measured by neuroimaging (30, 37).

Animal models are beginning to emerge investigating the neurological effects and possible mechanisms of perinatal glucocorticoids (10, 17, 18). Although caution is necessary when extrapolating from animal models to the clinical setting, there is very little variation in the general sequence of brain growth between laboratory animals and humans (14); the main differences being the timing of events that lead to spurts in brain growth. In humans, neuronal proliferation is completed before the 24th week of gestation, exceptions being the cerebellum and dentate gyrus (14). After this gestational age, glia continue to proliferate and oligodendroglial maintain ongoing myelination, with a peak in brain growth occurring near term. In contrast to humans, rodents have their brain growth spurt after birth. It is estimated that in terms of brain growth rate, periventricular germinal matrix composition, neurochemical expression, electroencephalographic patterns, and synapse formation, at approximately postnatal *day* (PD) 7, the rat brain is roughly equivalent in development to that of a full-term human infant (38 to 40 wk postconception; Refs. 14, 15, 23). With the use of this model of cross-species neurodevelopment, the brain of a rat pup at birth (PD 1) corresponds to that of

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a human fetal brain at or near 22–24 wk gestation (15, 55). Extrapolating this brain growth velocity model further, the *PD 2* brain approximates that of a 26- to 27-wk-old human brain in terms of gross neurodevelopment, the *PD 3* brain corresponds with that of a 28- to 29-wk-old human brain, and the *PD 6* brain corresponds with that of a 35- to 36-wk-old human brain. Given these developmental correlations, the neonatal rat pup provides a model in which to investigate effects of a prolonged, tapering course of neonatal Dex on the developing CNS.

The present study was designed to develop a rat model system in which to study long-term effects of a prolonged, tapering course of neonatal Dex administration. Unlike other studies, where single doses of Dex were given at particular postnatal ages (6, 16), our objectives were twofold: 1) to provide Dex during a postnatal age in the rat that corresponds to the neurodevelopmental time point at which human premature infants receive prolonged Dex therapy and 2) to provide Dex in tapering doses (between *PD 3* and *PD 6*). The specific goal of this model is to more closely mimic glucocorticoid protocols provided in the neonatal intensive care setting where 42-day (6 wk) courses are still administered.

## METHODS

**Animals.** Adult Sprague-Dawley rats (Charles Rivers, Wilmington, MA) were housed in our animal unit and maintained in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All animals were kept under constant temperature ( $25 \pm 2^\circ\text{C}$ ) and photoperiodicity (14:10-h light-dark cycle), and they were provided with food and water ad libitum.

With the use of the trio mating system (2 females:1 male), 20 female rats were mated. Females were then housed in pairs until estimated gestational *day 18*, at which point they were housed individually. The day of birth was designated *PD 1*. On *PD 2*, each litter was sexed and culled to 12 pups (6 males:6 females) to ensure equality in nutrition and maternal care within litters. Pups were separated into three treatment groups on *PD 3*, with each group represented within one litter, to control for variations in maternal behavior. On *PD 8*, a male and a female pup from each treatment group were killed, reducing the litter size to six animals for the remainder of the study.

**Drug treatment.** As stated above, each litter was assigned to three treatment groups: handled controls (Han), saline-injected animals (Veh), and Dex-treated animals (Dex). All pups within each litter were removed from their mother and treated (or handled) between the hours of 1100 and 1300 for a period of 5 min. Animals in the Dex group received an intramuscular injection of Dex in tapering doses on *PD 3* through 6 (0.5 mg/kg on *PD 3*, 0.25 mg/kg on *PD 4*, 0.125 mg/kg on *PD 5*, and 0.05 mg/kg on *PD 6*; American Pharmaceutical Partners, Los Angeles, CA). Animals in the Veh group received equivalent volumes of intramuscular sterile saline as the Dex animals, and animals in the Han group received no injection but were handled during the same time period on *PD 3* through 6.

**Animal measurements.** Weight and length were recorded before handling or injection on *PD 3-6*, 8, 14, and 20 for each treatment group. Length was measured from the nose to the base of the tail (rump length) in each pup. Procedures for

testing neurological responses were derived and adapted from those reported by Altman and McCrady, Fox, and Wahlsten (2, 20, 50). Each pup was submitted to testing between the hours of 1100 and 1300 on *PD 7*, 14, and 20. Two investigators performed the evaluation of the responses of all animals using a 0–5 rating scale with a score of 5 corresponding to the mature response (Table 1). Measures used to examine neonatal neurodevelopment included posture, righting reflex, postural flexion and extension, vibrissa placing, forelimb and hindlimb placing, geotaxis, and bar hold. Physical maturity was measured by observing eye opening, ear opening, ear folding, fur development, and tooth eruption.

**Behavioral testing.** All behavioral testing occurred between the hours of 0700 and 1000. On *PD 21*, open-field behavior was assessed. Rats were placed in the center of a brightly illuminated  $70 \times 70 \times 31$ -cm Plexiglas box with the floor divided into 16 equal squares. Motor activity and behavioral measures were recorded during a 2-min test period. An animal was considered to have “crossed squares” when its entire body entered a new square. The amount of time spent rearing and grooming was counted sequentially throughout the test. Defecation and urination were also recorded.

Light-dark preference was tested on *PD 33*. The testing apparatus consisted of a covered  $30 \times 60 \times 30$ -cm Plexiglas shuttle-box with a stainless steel grid floor suspended above corncob bedding. Boxes were divided into two equal-sized compartments with a 12-cm-wide opening. The light compartment was constructed of white Plexiglas and was brightly illuminated; the dark compartment was constructed of black Plexiglas and was minimally lit. Each animal was placed in the dark compartment, and the amount of time elapsed before the animal entered the lit side was recorded. Locomotor activity as well as time spent in each compartment were monitored by photocells located on the wall of each box, with the number of photocell beams interrupted per unit time recorded with a microprocessor. Total testing time was 5 min.

**Adrenocortical response to novelty stress.** On *PD 33*, blood sampling was performed via the tail nick method at 15, 30, 60, and 120 min following exposure to the preference box, with a basal time point obtained before the procedure. Blood was collected in tubes containing EDTA and then spun at 2,000 rpm for 7 min. Serum was collected from the spun sample and stored at  $-20^\circ\text{C}$ .

Corticosterone was measured using a radioimmunoassay as previously described (48). Briefly, plasma samples were diluted (1:100) in 50 mM sodium phosphate buffer containing 2.5% bovine serum albumin at pH 7.5. Samples were heated to  $80^\circ\text{C}$  to separate corticosterone from the binding protein. The corticosterone antibody used (a gift from Huda Akil) cross-reacts 2.2% with cortisol and  $<1\%$  with other endogenous steroids. Tritiated corticosterone (Amersham, Arlington Heights, IL) was used as a radiolabeled trace. Bound [ $^3\text{H}$ ]corticosterone was separated from free ligand using a suspension of 2% charcoal and 0.2% Dextran. The detection limit of the assay is 1 pg/ml with intra- or intercoefficients of variation, 2 and 3%, respectively.

**Brain weights.** Brain weights were obtained during necropsy on *PD 8*, 23, and 35. Brains were then quick-frozen in liquid isopentane ( $-40^\circ\text{C}$ ) and stored at  $-80^\circ\text{C}$  for future processing and analysis.

**Statistical analysis.** Body weight, length, rate of growth, brain weight, hormonal values, and behavioral data were averaged across treatment groups and ages. Results were subject to ANOVA considering age, sex, and treatment simultaneously. Total and individual neurological scores were also averaged across treatment groups and ages, and these re-

Table 1. *Neurological exam rating scale*

Behavior	0	1	2	3	4	5
Posture	Full flexion	Partial flexion		Full extension	Partial extension	Normal
Eye opening	Closed		Occasional opening	Occasional opening		Fully open
Ear opening	Closed		Occasional opening	Occasional opening		Fully open
Ear folding	Barely visible	Completely folded		Partially folded		Fully open
Fur development	No fur		Fine hairs	Partial fur	Mostly fur	Full
Maxillary eruption	No teeth	Initial maxillary	Partial maxillary	Partial maxillary	Mostly full	Maxillary full
Mandibular eruption	No teeth	Initial mandibular	Partial mandibular	Partial mandibular	Mostly full	Mandibular full
Ear twitch response	No response	Weak response		Moderate response		Strong response
Righting reflex	On side full flexion	Partial flexion	Extension predominates	Extension predominates	Partial extension	Normal position
Postural flexion	Absent		Partial	Moderate		Present
Postural extension	Absent		Partial	Moderate		Present
Vibrissa placing	No response	Weak response		Moderate response		Strong response
Forelimb placing	No response	Weak response		Moderate response		Strong response
Hindlimb placing	No response	Weak response		Moderate response		Strong response
Geotaxis	No response	Pivoting predominates		Turns 180°		Strong response
Bar hold	No grasp	Weak grasp		Moderate grasp		Full grasp
Grasp reflex forelimb	No grasp	Weak grasp		Moderate grasp		Full grasp
Grasp reflex hindlimb	No grasp	Weak grasp		Moderate grasp		Full grasp
Cross extensor	Absent	Weak		Moderate		Strong

To evaluate responses of rat pups to a neurological test battery, a 0 score was given for complete immature response on exam or immature physical development. A score of 5 corresponds to the completely mature response or physical maturation. Neonatal neurodevelopment parameters measured were posture, righting reflex, postural flexion and extension, vibrissa placing, forelimb and hindlimb placing, geotaxis, and bar hold. Physical maturity parameters measured were eye opening, ear opening, ear folding, fur development, and tooth eruption.

sults were subject to analysis using the Kruskal-Wallis test of nonparametric values. For all tests, when sex differences were determined to be nonsignificant, the data were collapsed across the respective variable. The level of significance was set at  $P \leq 0.05$ , and post hoc comparisons were done using Fisher's protected least-significant differences tests.

## RESULTS

**Growth measurements.** Females weighed significantly less than males in each of the treatment groups at all ages (*PD* 4–8,  $n = 40$ ,  $P < 0.001$ ; *PD* 14 and 20,  $n = 20$ ,  $P < 0.05$ ), and Dex-treated animals weighed significantly less than the comparison groups on *PD* 4–8 ( $n = 80$ ), *PD* 14 ( $n = 40$ ), and *PD* 20 ( $n = 40$ ,  $P < 0.0001$ ; Fig. 1A). Rump length was significantly less in Dex-treated animals on *PD* 7 ( $n = 80$ ), *PD* 14 ( $n = 40$ ), and *PD* 20 ( $n = 40$ ,  $P < 0.0001$ ; Fig. 1B). Dex-treated pups gained weight at a slower rate than comparison animals for up to 2 wk after treatment was discontinued ( $n = 40$ ,  $P < 0.0001$ ). A significant difference was found in the rate of length gain only during the first week of life ( $n = 80$ ,  $P < 0.0002$ ).

**Brain weights.** Brain weights in Dex-treated animals were reduced on *PD* 8 and 23 compared with both Veh and Han controls. However, this effect only reached

significance between Dex and Veh animals ( $n = 10$ ,  $P < 0.05$ ; Fig. 2A). In contrast to the differences observed in total brain weights between groups, Dex-treated animals maintain a higher ratio of brain weight to body weight compared with both Veh and Han groups at *PD* 8 and 23 ( $n = 10$ ,  $P < 0.05$ ; Fig. 2B). Interestingly, on *PD* 35, the Veh group had a significantly lower brain weight-to-body weight ratio than either the Dex or Han groups ( $n = 10$ ,  $P < 0.05$ ; Fig. 2B).

**Neurological examination.** For global neurological assessment, a cumulative neurological test score for each treatment group was assessed (Table 1). Although Dex-treated animals exhibited a reduced total neurological score compared with the Veh and Han groups on *PD* 7 (mean total neurological scores  $\pm$  SE: Dex  $44.53 \pm 0.572$ ; Han  $47.79 \pm 0.392$ ; Veh  $47.03 \pm 0.530$ ;  $n = 80$ ,  $P < 0.001$ ), no significant difference on the total neurological score was noted on *PD* 14 and 20.

Differences observed on the neurological exam on *PD* 7 included delays in physical maturation (ear unfolding) and neurodevelopment (immature posture, forelimb placing, and postural extension reflexes) in Dex-treated animals compared with controls ( $n = 80$ , Han  $P < 0.05$ ; Veh  $P < 0.01$ ; Fig. 3). On *PD* 14, Dex-treated

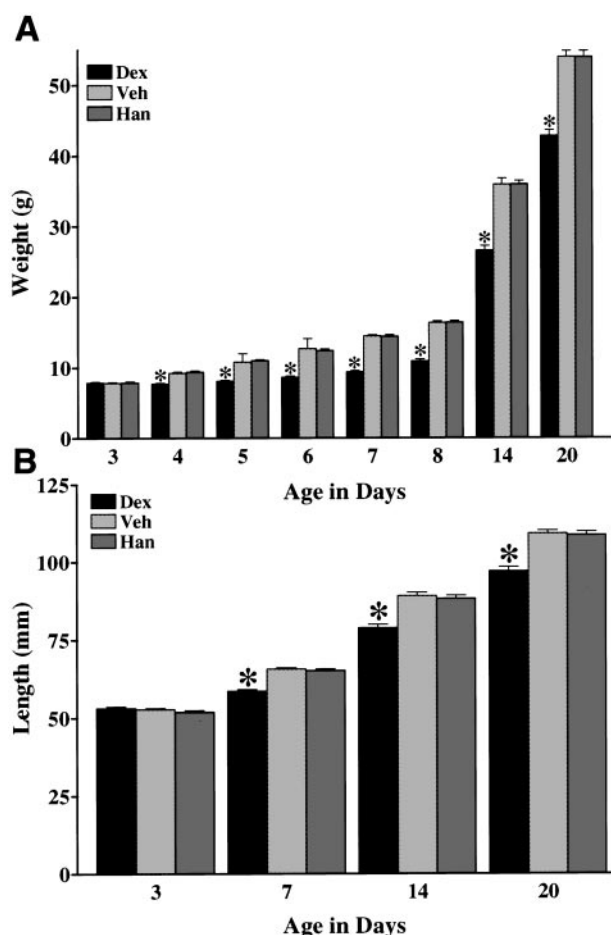


Fig. 1. Effect of dexamethasone (Dex) treatment on increases in weight (A) and length (B). Each vertical bar represents the means  $\pm$  SE for each group. Dex-treated rats weighed less and maintained a shorter nose-to-rump length than vehicle (Veh) and control (Han) animals at early ages [postnatal day (PD) 4, 5, 7, and 8,  $n = 80$ ; PD 14 and 20,  $n = 40$ ;  $*P < 0.0001$  for all groups].

animals also exhibited delays in physical maturation (ear folding and fur development) and neurodevelopment (posture and vibrissa reflex;  $n = 40$ ; Han  $P < 0.05$ ; Veh  $P < 0.05$ ; Fig. 4). Interestingly, Dex-treated animals exhibited a marked acceleration in eye opening at this postnatal age ( $n = 40$ ,  $P \leq 0.0001$ ; Fig. 4). By PD 20, minor delays of physical maturation and posture persisted in Dex-treated animals, with only delayed fur development in Dex-treated animals reaching significance ( $n = 40$ ,  $P \leq 0.05$ ; Fig. 5).

**Behavioral testing.** In open-field testing, there were no significant sex differences observed. No significant differences between Dex-treated animals and comparison groups on PD 21 were observed in the number of squares crossed, time spent rearing, or the amount of defecation and urination. There was, however, a trend effect for the number of squares crossed, with Dex-treated animals crossing fewer squares than the control groups (mean number of squares crossed  $\pm$  SE: Dex  $9.98 \pm 1.60$ ; Veh  $13.95 \pm 1.86$ ; Han  $10.43 \pm 1.2$ ).

On PD 28, Dex-treated animals were less active in both dark and light environments compared with the

control groups ( $n = 25$ ,  $P < 0.05$ ; Fig. 6A). There were no significant differences in emergence time to the light compartment or total time spent in either the light or dark compartments among the three groups. No sex differences were observed.

**Adrenocortical response to novelty stress.** On PD 8, basal corticosterone levels in Dex-treated animals were suppressed when compared with Veh and untreated controls (mean serum corticosterone concentration in  $\mu\text{g/dl} \pm \text{SE}$ : Dex  $0.175 \pm 0.05$ ; Veh  $0.660 \pm 0.16$ ; Han  $0.552 \pm 0.17$ ;  $n = 19$ ,  $P < 0.05$ ). No differences in basal corticosterone levels were noted between groups on PD 23 (mean serum corticosterone concentration in  $\mu\text{g/dl} \pm \text{SE}$ : Dex  $1.43 \pm 0.39$ ; Veh  $2.16 \pm 0.79$ ; Han  $1.78 \pm 0.52$ ;  $n = 8$ ) or on PD 33 (mean serum corticosterone concentration in  $\mu\text{g/dl} \pm \text{SE}$ : Dex  $0.69 \pm 0.45$ ; Veh  $0.79 \pm 0.42$ ; Han  $1.02 \pm 0.37$ ;  $n = 10$ ). On PD 33,

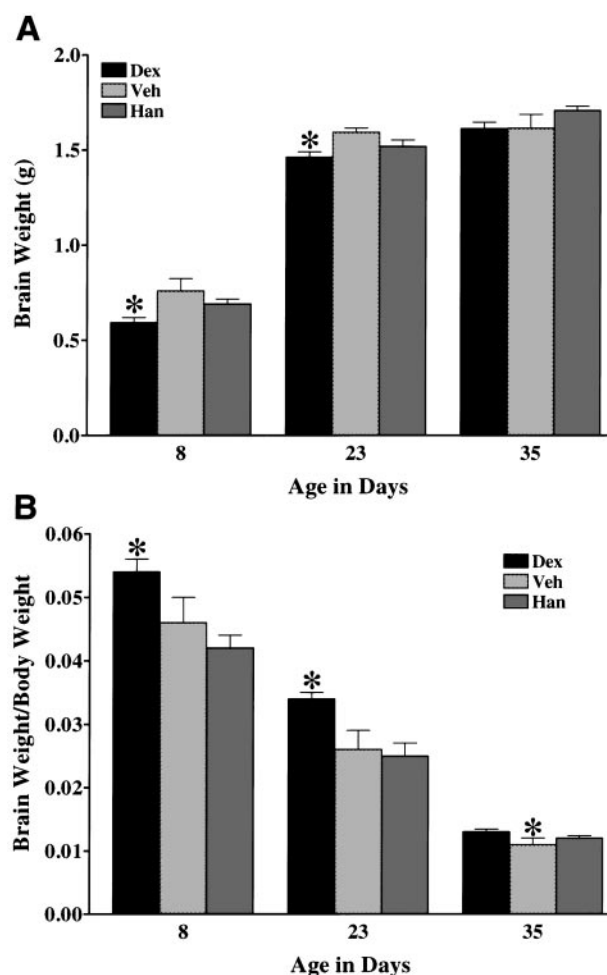


Fig. 2. Effect of Dex treatment on brain weight (A). Each vertical bar represents the means  $\pm$  SE for each group. Total brain weight in rats exposed to Dex was decreased compared with Veh animals ( $n = 10$ ,  $*P < 0.05$ ) on PD 8 and 23. No difference was observed between groups at PD 35. When examining brain weight corrected for changes in body weight (B), Dex-treated animals had a higher brain-to-body weight ratio compared with both Veh and Han groups at PD 8 and 23 ( $n = 10$ ,  $*P < 0.05$ ). At PD 35, the Veh-treated animals differed from the Han and Dex groups, showing a decreased brain to body weight ratio ( $n = 10$ ,  $P < 0.05$ ).

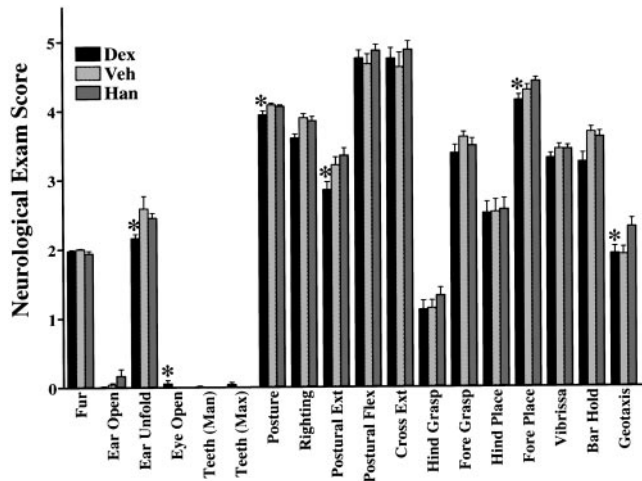


Fig. 3. Effect of Dex treatment on neurological exam at 7 days of age ( $n = 80$ ). Each vertical bar represents the means  $\pm$  SE for each group. Significant differences observed in neurological exam on PD 7 included delayed ear unfolding, immature posture, delayed forelimb placing, and postural extension reflexes in Dex-treated animals. Dex-treated animals also appeared less mature with the bar hold contrasted with the Han and Veh groups (reaching trend level at  $P = 0.07$ ). Ext, extension; flex, flexion; hind, hindlimb; and fore, forelimb. ( $*P < 0.05$ ).

in response to novelty stress, Dex-treated animals demonstrated an early blunted corticosterone peak, followed by an early termination to baseline (Fig. 6B). There were no sex differences noted in basal corticosterone levels at all ages examined or in response to novelty stress.

## DISCUSSION

In the present study, we have investigated early and long-term effects of a Dex treatment regimen given

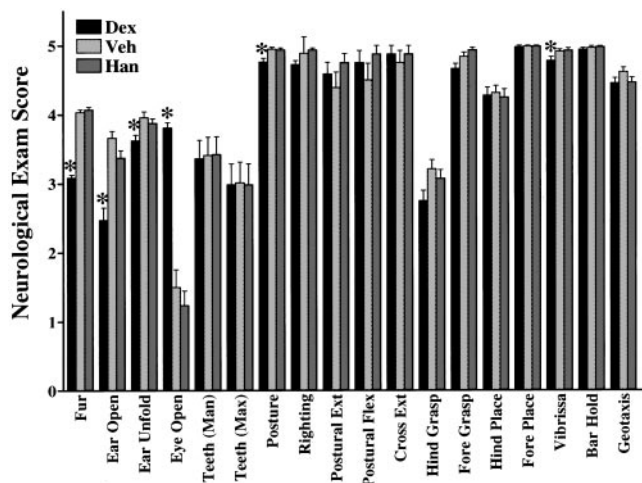


Fig. 4. Effect of Dex treatment on neurological exam at 14 days of age ( $n = 40$ ). Each vertical bar represents the means  $\pm$  SE for each group. Significant differences observed in neurological exam on PD 14 include delays in ear opening and unfolding, fur development, posture, and vibrissa reflex in the Dex-treated animals. A trend level effect was reached for forelimb and hindlimb grasp and the righting reflex ( $P < 0.10$ ). Dex-treated animals also exhibited a marked acceleration in eye opening. ( $*P < 0.05$ ).

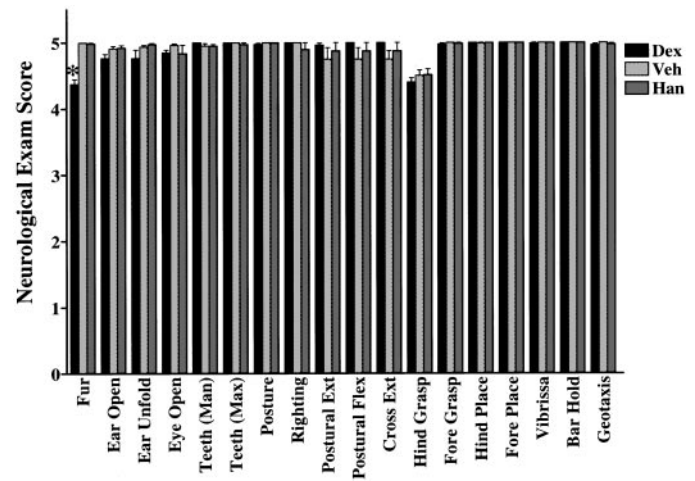


Fig. 5. Effect of Dex treatment on neurological exam at 20 days of age ( $n = 40$ ). Each vertical bar represents the means  $\pm$  SE for each group. Except for minor delays in fur and ear development, the neurological exam findings were not different between groups on PD 20. ( $*P < 0.05$ ).

during the first week of life in the infant rat. Unlike previous studies that have investigated the effects of neonatal exposure to glucocorticoids in rats (4, 22, 49), we administered tapering doses of Dex between PD 3 and 6 in an attempt to mimic a prolonged 42-day treatment regimen commonly used in the neonatal intensive care setting. The timing of drug administration is critical in our rat model, and it corresponds to the third trimester of human pregnancy, a period of growth and development that renders a brain highly vulnerable to insult in the human neonate (15). We found that Dex treatment induced significant decreases in length and weight gain in the rat pup that persisted for up to 2 wk after treatment. Dex-treated animals also demonstrated differences in neurological development at PD 7 and 14 but not at PD 20 upon weaning. Interestingly, although absolute brain weights were found to be significantly less in Dex-treated animals compared with Han controls on PD 8, 23, and 35, this relationship disappears when corrected for differences in somatic weight. Beyond these somatic and CNS observations, we found that in early adolescence, Dex-treated animals demonstrate altered behavioral responses to a novel environment. A blunted corticosterone response to novelty stress was also observed in adolescent animals exposed to Dex during the early neonatal period. These findings point to the long-lasting effects of Dex administration during a time of peak brain development, even when a tapering dose regimen is implemented to minimize adverse effects from glucocorticoid exposure.

**Somatic growth measurements.** Our study suggests that a regimen of decreasing Dex exposure early in life has a lasting impact on somatic growth. One possible explanation for the decreased somatic growth observed in the Dex-treated pups is inadequate nutritional intake during the postnatal period. Although we cannot exclude this possibility, we presume that the somatic and brain weight deficits observed in Dex-treated ani-

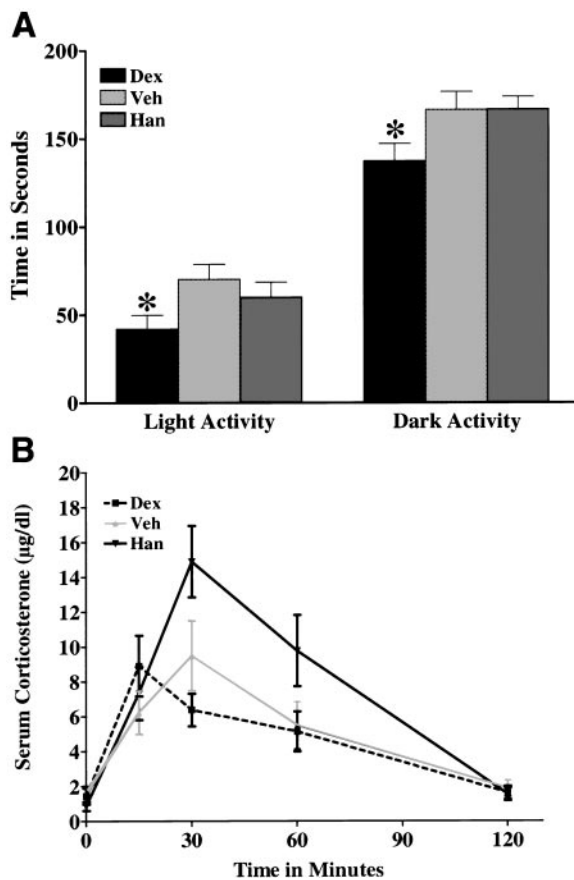


Fig. 6. Effect of Dex treatment on testing of place preference (A) and serum corticosterone in response to a novel stress (B). Each vertical bar represents the means  $\pm$  SE for each group. Dex-treated animals were less active in a test of adaptation to a novel environment. Significant differences were found in both light and dark motor activity when tested on PD 28 ( $n = 25$ ,  $*P < 0.05$ ). In response to a novel stress, animals in the Dex group demonstrate a blunted corticosterone peak, followed by early termination to baseline (B).

mals are primarily due to direct Dex effects on catabolism and tissue accretion. Other investigators have clearly shown that the dam spends more time giving nutrition, stimulation, and warmth to a litter that is perceived to have poor health (29, 53). Dams readily recognize disparities in their young. Some factors that appear to heighten maternal sensitivity to their young are decreased weight, reduction in motor movement, and enhanced vocalizations (12, 43). In our study, Dex-treated animals were easily identified visually, and abnormal results found in the neurological examination are within the repertoire of pup behaviors that would provoke nurturing from the dam (43). In support of Dex-induced catabolic effects on weight gain, previous reports have demonstrated that Dex alters metabolic pathways. In a setting of sufficient caloric intake, Dex has been shown to increase protein catabolism and prevent adequate growth (8, 27, 42, 52). For example, in a piglet model, Weiler and colleagues (52), using an oral dose of Dex at  $0.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  for 15 consecutive days, demonstrated that Dex rapidly induces protein catabolism beyond the capacity for an anabolic

state, resulting in an elevation of blood urea nitrogen and a reduction of circulating total protein. These findings correlated with functional measures of reduced growth and lean body mass (52). Leucine kinetic studies in Dex-treated human infants (46) and piglets (24) further support this argument. These studies have established that, rather than an inadequate nutritional intake that would preclude anabolism during and after Dex exposure, the major contributing factor to the failure to thrive observed in infants given Dex is the elevation in protein catabolism. In energy expenditure studies of human infants with chronic lung disease, reduced growth in Dex-treated infants could not be explained by increased energy expenditure or decreased caloric intake (21, 27, 42). It should be noted that in the present study, a significant weight difference is observed between Dex-treated and control animals on PD 4, only 24 h after the first Dex dose. This observation lends support to the notion that decreased nutritional intake, particularly fluid and free water, may play a more acute role in weight disparity immediately after Dex exposure. However, given the body of evidence supporting the scenario of increased catabolism and decreased accretion of tissue, nutritional intake seems unlikely to be the sole explanation for decreased somatic growth (27).

**Neurological development.** Our data also reveal that Dex-treated animals experience transient variations in neurological development compared with control animals. On PD 7, Dex-treated animals exhibited mild differences in the gross neurological exam, which included immature posture at rest and delayed forelimb placing and postural extension reflexes. Differences in neurodevelopment were also observed on PD 14, with an abnormal response to vibrissa placing and a persistence of the forelimb and hindlimb grasp reflexes. As a functional unit, vibrissa maintain an extensive representation in the sensory motor cortex, with considerable modulatory input from cerebellar and vestibular systems (51, 54). It is important to note that gross neurological deficits observed early on in our Dex-treated pups were no longer evident by PD 20, suggesting that the pathways relevant to organization of reflexes and behavior assessed on our exam reached acceptable criteria of normality by the time of weaning.

Primitive reflexes appear and disappear in defined sequences during specific periods of development. Absence of an expected primitive reflex, or persistence beyond an expected time of extinction, typically indicates severe dysfunction within the CNS (32). Little data exist pertaining to specific neurological processes that might impinge on the organization of these reflexes in the rodent. However, it is known that an "immature posture" reflex consists of a predominance of flexion that is characteristic in the first 2 days of life. Postural flexion inherently inhibits gross motor actions, such as crawling and rooting activities. Thus postural flexion beyond 3 days of life signifies generalized CNS dysfunction, which may or may not be permanent (20). In contrast, strong stereotyped reflexes and limb placement reactions are expected to be evi-

dent after *PD* 3, persisting to *PD* 9. These reflexes provide the organism with important information concerning body orientation. Delays in development of such complicated behavioral reflexes in Dex-treated pups would indicate perturbations or alterations in multiple integrated circuitry, including contributions from sensory regions, major developing motor regions, and the cerebellum (20). A growing body of evidence is emerging to support the notion that early neurodevelopmental delays observed in animals treated with a prolonged course of Dex during early neonatal life may be due primarily to an inhibition of the normal myelination processes, including *in vitro* and *in vivo* animal studies and evaluations of very low birth weight human infants treated with Dex (18, 30, 35, 41, 45). Morphological evaluations to ascertain changes in myelination or other gross neuroanatomic parameters were not performed in the present study, and it remains unclear at present whether permanent CNS changes have occurred that are not evident on our neurological exam.

**Brain weights.** Dex treatment correlated with low brain weights in our young animals, persisting into early adolescence (*PD* 35). Interestingly, when corrected for variations in somatic weight, brain weights did not differ between groups. Although such a discrepancy provides an argument that catabolism and nutrition play a major role in brain size reduction, the fact that Dex-treated animals have smaller brains than Veh and Han animals still remains. Ferguson and Holson (18), in fact, report reduced brain weight in 28-day-old animals treated with Dex on *PD* 7. Similar findings have also been reported in human clinical studies (30). Murphy and colleagues (30) have recently reported a 30% reduction in cerebral tissue volume on neuroimaging in premature infants treated with Dex compared with untreated, age-matched controls.

From a mechanisms perspective, decreased brain weight in Dex-treated animals is not unexpected in light of previous studies of prenatal Dex exposure, where neuronal maturation, replication, differentiation, programmed cell death, and organization of synaptic connections were clearly affected in brain regions that are at the peak of neuronal mitosis (31, 36, 55). Although the formation of new neuronal cells in the brain is limited, other brain cell types are actively dividing and differentiating postnatally at all levels of the CNS in many species, including primates (5). During the time frame of postnatal myelination and differentiation, Dex may be exerting damaging effects on the developing CNS, with reduction in brain weight as one outcome. Interestingly, in a randomized study of premature infants treated with Dex, an increased incidence of cerebral palsy was observed at 1 yr of age (37), an outcome that has been attributed primarily to white matter damage (32). Although in our study low brain weights also corresponded with somatic weight loss, one cannot rule out the possibility that Dex treatment interferes with neurogenesis within specific vulnerable brain structures.

**Adrenocortical response to novelty stress.** Our evaluation of the LHPA axis revealed that basal corticosterone levels were suppressed by Dex, but only in the *PD* 8 Dex-treated animals. This finding is of interest given that the developing rat already exhibits a reduced basal corticosteroid level and a minimal corticosteroid response to stressful stimuli between *PD* 4 and 14 [stress hyporesponsive period (SHRP)]. During the SHRP, under circumstances of stress, the ACTH and corticosterone responses are limited in magnitude and sustained, indicating both an immature activation of the stress axis and an immature termination of the stress response (44). The termination of the stress response is mediated through corticoid receptors in the developing CNS [type I or mineralocorticoid receptor (MR) and type II or glucocorticoid receptor (GR); Refs. 38–40]. The abundance and pattern of distribution of MR and GR mRNA are individually distinct during development in the hippocampus, with highest signal intensity found on *PD* 10. Beyond this age, as GR mRNA expression diminishes, MR mRNA expression continues to evolve, acquiring its adult-like distribution after *PD* 28 (48). Thus, during the early SHRP and up to ~10 days of age, the hippocampus is acquiring the greatest number of GRs that would become important for the reactive negative feedback action of the LHPA axis, as has been demonstrated by van Oers and colleagues (47). Because Dex has a prolonged half-life and higher affinity for GR compared with corticosterone, one can conclude that the low basal corticosterone levels observed in our Dex-treated animals on *PD* 8 are a result of prolonged pituitary and brain GR occupation that consequentially enhances negative feedback at this age.

In the present study, we observed a blunted corticosterone response to novelty stress, with a prompt return to basal levels in the Dex-treated animals at *PD* 33. This is in agreement with Felszeghy and colleagues (16) who reported a suppressed elevation of both ACTH and corticosterone concentrations in response to restraint stress in adult rats that were exposed to a fixed dose of Dex on *PD* 1, 3, and 5. Our findings also suggest that there is an immediate and long-lasting effect of Dex on the developing LHPA axis, even though Dex was administered in tapering doses. Additionally, Dex-treated animals were less active in both dark and light environments compared with the control groups on *PD* 33, suggesting a possible anxious state in these young animals while displaying a blunted corticosterone response.

In conclusion, early exposure to glucocorticoids has long-lasting effects on neurodevelopment and neuroendocrine function even when drug doses are deliberately reduced to decrease adverse events. In particular, effects on stress responsiveness and behavior are dissociated and not evident until adolescence. These findings raise concerns about maladaptive behavioral strategies that may be subtle and not recognizable until later in development. Such effects may have important implications on learning, mood, and, ultimately, quality of life in an organism.

### Perspectives

Nationwide, neonatal intensive units provide medical care to infants born as early as 24 wk gestation. Dex treatment is readily used in these newborns with the purpose of reducing time of hospitalization. Our study mimics the equivalent of a 42-day Dex taper commonly used for this purpose. Our findings, while underscoring the somatic effects of steroids that have been reported in other studies, also contribute new information regarding long-term effects of Dex on stress response and behavior later in life. In early adolescence, Dex-treated animals are less active in both dark and light environments compared with controls, a behavioral response suggestive of an anxious state in these animals. Despite this state of increased anxiety, the adrenocortical response to stress is blunted, creating a dissociation between the behavioral and physiological responses that may be viewed as maladaptive. Given that adequate glucocorticoid activation is vital for learning [memory acquisition is dependent on optimal exposure to elevated glucocorticoid levels (28)], the results of this present study should raise concern with regards to the neurodevelopmental repercussions a prolonged steroid course may have on the premature human infant. The exposed infant's ability to mobilize energy resources and to promote anabolic processes during the period of Dex exposure is an acute matter. Perhaps of greater concern are the long-term consequences on behavior, particularly on anxiety and attention, which may interfere with later learning and adjustment in the early school years. Our findings point to the need to study mechanisms of cognitive and behavioral effects of treating premature infants with Dex during their early postnatal life.

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