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Critical Prenatal and Postnatal Periods for Persistent Effects of Dexamethasone on Serotonergic and Dopaminergic Systems

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Glucocorticoid administration to preterm infants is associated with neurodevelopmental disorders. We treated developing rats with dexamethasone (Dex) at 0.05, 0.2, or 0.8 mg/kg, doses below or spanning the range in clinical use, testing the effects of administration during three different stages: gestational days 17–19, postnatal days 1–3 or postnatal days 7–9. In adulthood, we assessed the impact on synaptic biomarkers for serotonin (5-hydroxytryptamine (5HT)) systems. Across all three regimens, Dex administration evoked upregulation of cerebrocortical $5HT_{1A}$ and $5HT_2$ receptors and the presynaptic 5HT transporter, greatest for $5HT_{1A}$ receptors. The effects were fully evident even at the lowest dose. In contrast, 5HT levels in the cerebral cortex and hippocampus showed disparate patterns of temporal sensitivity, with no change after gestational treatment, an increase with the early postnatal regimen, and a decrease with the later postnatal exposure. None of the changes in 5HT concentrations were offset by adaptive changes in the fractional 5HT turnover rate. Furthermore, the critical period of sensitivity seen for 5HT levels differed from that of dopamine even within the same brain region. These findings suggest that developmental exposure to Dex during the critical neurodevelopmental period corresponding to its use in preterm infants, elicits selective changes in 5HT and dopaminergic synaptic function over and above its effects on general aspects of neural cell development, below the threshold for somatic growth impairment, and even at doses below those used clinically. Accordingly, adverse neurobehavioral consequences may be inescapable in glucocorticoid therapy of preterm infants.
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INTRODUCTION

Glucocorticoids are now the consensus treatment for preterm labor occurring between the 24th and 34th weeks of gestation (Gilstrap *et al*, 1995). These agents, principally dexamethasone (Dex), are now used in nearly one in every ten pregnancies in the US, and consequently, although thousands of preterm infants are spared the adverse consequences of respiratory distress syndrome, *hundreds* of thousands of infants actually receive treatment (Matthews *et al*, 2002). Additionally, the use of multiple glucocorticoid courses is common (Crowther and Harding, 2003; Dammann and Matthews, 2001), despite the likelihood of subsequent metabolic, cardiovascular and behavioral anomalies (Barrington, 2001; Seckl, 2001; Shinwell *et al*, 2000; Trautman *et al*, 1995; Yeh *et al*, 2004). Recent reviews point out the long-term consequences of the use of antenatal steroids (Blackmon *et al*, 2002; Coe and Lubach, 2005; Newnham, 2001; Raff, 2004; Seckl, 2004) but isolating an explicit glucocorticoid effect in human populations is confounded by the comorbidities and interventions that are characteristic of preterm labor; as just one example, terbutaline, which is typically given to arrest uterine contractions (Lam *et al*, 1998) is itself a developmental neurotoxicant (Rhodes *et al*, 2004; Slotkin *et al*, 2003).

Accordingly, there is a critical need for animal studies specifically designed to assess the consequences of glucocorticoids used within the developmental context of preterm labor. Heretofore, most reports have focused on treatments that produce persistent stunting of somatic growth, outright cerebral atrophy and endocrine disruption (Bohn, 1984; Fuxe *et al*, 1994, 1996; Gilad *et al*, 1998; Gould *et al*, 1997; Maccari *et al*, 2003; Matthews, 2000; Matthews *et al*, 2002; McEwen, 1992; Meaney *et al*, 1996; Weinstock, 2001; Welberg and Seckl, 2001). To bridge the gap, we recently performed studies in developing rats, showing that Dex treatment, even at doses that lie below those used in the therapy of preterm delivery and that do not compromise long-term somatic growth, nevertheless disrupts neural cell acquisition, indices of neuritic outgrowth, synaptic activity and cell signaling involved in trophic regulation of forebrain development (Kreider *et al*, 2005a). These effects

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were associated with long-term changes in cognition and motor activity (Kreider *et al*, 2005b), resembling those seen in models of prenatal stress (Bowman *et al*, 2004; Dean *et al*, 2001; Felszeghy *et al*, 2000; Muneoka *et al*, 1997). Interestingly, the persistent effects on cholinergic synaptic function showed regional selectivity and critical periods of vulnerability that differed from the general effects on neural cell acquisition and development (Kreider *et al*, 2006). Indeed, indices of synaptic function were disrupted far more extensively than general architectural biomarkers, representing interference with the functioning of a variety of cholinergic and noncholinergic G-protein-coupled receptors, superimposed on presynaptic hyperactivity. These findings suggest that morphological evaluations of the consequences of developmental exposure to Dex (Bohn, 1984) grossly underestimate the vulnerability of the developing brain and that the misprogramming of synaptic function may represent one of the major targets responsible for neurobehavioral anomalies.

In the current study, we evaluated the targeting of serotonergic (5-hydroxytryptamine (5HT)) systems. In the adult, glucocorticoids play a key role in the regulation of 5HT receptor expression and synaptic function, contributing to many of the attributes of affective disorders (Aghajanian *et al*, 1993; Young, 1994) and therefore we hypothesized that 5HT pathways were a likely target for anomalous programming of synaptic function consequent to prenatal or early neonatal Dex treatment. We administered Dex to rats during defined perinatal periods corresponding to phases of human neurodevelopment in the second to early third trimester, the period which glucocorticoids are most likely to be used in preterm infants (Dobbing and Sands, 1979; Gilstrap *et al*, 1995; Kreider *et al*, 2005a, 2006; Rodier, 1988): gestational days (GD) 17–19, postnatal days (PN) 1–3 and PN7-9. For each regimen, we examined doses below (0.05 mg/kg) or within the therapeutic range (0.2 or 0.8 mg/kg), a strategy adopted from our earlier work on architectural and cholinergic biomarkers (Kreider *et al*, 2005a, 2006), and focused on the cerebral cortex and hippocampus, the regions displaying the largest and most persistent effects on architectural, cholinergic and synaptic signaling indices (Kreider *et al*, 2006). In addition, a number of studies point to sexually dimorphic neurochemical and behavioral effects of developmental glucocorticoid exposure (Bowman *et al*, 2004; Gerardin *et al*, 2005; Kreider *et al*, 2005a, b, 2006; Rieger *et al*, 2004), so we contrasted the long-term effects in males and females.

Our assessments focused on expression of the presynaptic 5HT transporter, which is responsible for regulating the concentration of 5HT in the synapse (Cooper *et al*, 1996) and two 5HT receptor subtypes, $5HT_{1A}$ and $5HT_2$, that are known to be affected in human depression (Arango *et al*, 2001; Fujita *et al*, 2000; Yatham *et al*, 1999, 2000). In addition, we evaluated 5HT levels and turnover. The latter is an index of presynaptic neuronal activity (Slotkin *et al*, 2000; Xu *et al*, 2001) and was assessed using the metabolite ratio, that is, the proportion of 5-hydroxyindoleacetic acid (5HIAA) to 5HT. Finally, we contrasted the effects on 5HT levels and turnover with those for dopamine (DA), in order to determine whether different types of synapses within the same brain region can undergo separable programming of activity by developmental Dex exposure. DA and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were evaluated and again, the turnover was calculated by the metabolite ratio method (Slotkin *et al*, 2000; Xu *et al*, 2001).

METHODS

Animal Treatments

All studies were performed in accordance with the *Declaration of Helsinki* and with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health. Timed-pregnant Sprague–Dawley rats (Charles River, Raleigh, NC) were housed individually and given free access to food and water. For studies of gestational Dex exposure, dams received daily subcutaneous injections of Dex phosphate (Sigma Chemical Co., St Louis, MO) at doses of 0.05, 0.2, or 0.8 mg/kg on GD17-19, whereas controls received equivalent volumes (1 ml/kg) of isotonic saline vehicle. On the day after birth, all pups were randomized within their respective treatment groups and redistributed to the nursing dams, maintaining a litter size of 10 to ensure standard nutrition. Randomization was repeated every 3–4 days and in addition, dams were rotated among litters to obviate any differences in maternal caretaking. Crossfostering does not alter the developmental effects of Dex, nor does fostering of normal pups by Dex-treated dams produce apparent treatment effects in controls (Nyirenda *et al*, 2001). For studies of the effects of postnatal Dex treatment, pups were given 0, 0.05, 0.2, or 0.8 mg/kg on PN1-3 or PN7-9 and the same randomization procedures were followed. Animals were weaned on PN21.

On PN60, animals were decapitated, the cerebellum was removed and the forebrain was separated from the brainstem by a cut rostral to the thalamus, after which the hippocampus was dissected and the striatum was removed to leave the cerebral cortex as the remainder, which was then divided down the midline to give separate samples of left and right cortex. Brain regions were frozen in liquid nitrogen and stored at $-45^{\circ}C$. Each treatment group consisted of six males and six females, with each animal derived from a different litter.

5HT Receptors and Transporter

The samples of the left cerebral cortex were thawed and homogenized (Polytron, Brinkmann Instruments, Westbury, NY) in ice-cold 50 mM Tris (pH 7.4), and the homogenates were sedimented at 40 000 *g* for 15 min. The pellets were washed by resuspension (Polytron) in homogenization buffer followed by resedimentation, and were then dispersed with a homogenizer (smooth glass fitted with Teflon pestle) in the same buffer. Two radioligands (PerkinElmer Life Sciences, Boston, MA) were used to determine 5HT receptor binding (Aldridge *et al*, 2003, 2004): 1 nM [^{3}H] 8-hydroxy-2-(di-*n*-propylamino)tetralin (specific activity, 135 Ci/mmol) for $5HT_{1A}$ receptors, and 0.4 nM [^{3}H] ketanserin (specific activity, 63 Ci/mmol) for $5HT_2$ receptors. For $5HT_{1A}$ receptors, incubations lasted for 30 min at $25^{\circ}C$ in a buffer consisting of 50 mM Tris (pH 8), 0.5 mM $MgCl_2$ and 0.5 mM sodium ascorbate; 100 μM 5HT

(Sigma) was used to displace specific binding. For $5HT_2$ receptors, incubations lasted 15 min at 37°C in 50 mM Tris (pH 7.4) and specific binding was displaced with 10 μM methylsergide (Sandoz Pharmaceuticals, E. Hanover, NJ). Incubations were stopped by the addition of excess of ice-cold incubation buffer (without radioligand or displacing agent) and the labeled membranes were trapped by rapid vacuum filtration onto glass fiber filters that were presoaked in 0.15% polyethyleneimine. The filters were then washed twice with 3 ml of incubation buffer and radiolabel was determined. For binding to the presynaptic 5HT transporter (Aldridge *et al*, 2003, 2004), the membrane suspension was incubated in a buffer consisting of 50 mM Tris (pH 7.4), 120 mM NaCl, and 5 mM KCl, with addition of 85 pM [^{3}H] paroxetine (specific activity 19.4 Ci/mmol; PerkinElmer) with or without 100 μM 5HT to displace specific binding. Incubations lasted 120 min at 20°C.

5HT and DA Levels and Turnover

The samples of the right cerebral cortex and the hippocampus were thawed and homogenized in ice-cold 0.1 M perchloric acid and sedimented for 20 min at 40 000 *g*. The supernatant solution was collected and aliquots were used for analysis of 5HT, 5HIAA, DA, HVA and DOPAC by HPLC with electrochemical detection (Slotkin *et al*, 2000; Xu *et al*, 2001). Concurrently run standards, containing each of the neurotransmitters and metabolites (Sigma), were used to calculate the regional concentration of each neurochemical. DOPAC and HVA levels in the hippocampus were too low for accurate measurement, so only 5HT levels and turnover are reported for that region. Turnover values for 5HT and DA systems were then determined as the ratio of metabolites to the native neurotransmitter: 5HIAA/5HT and (DOPAC + HVA)/DA, respectively.

Data Analysis

Data are presented as means and SE. Differences between groups were first assessed by a global analysis of variance (ANOVA) (data log-transformed because of heterogeneous variance), incorporating all factors: treatment regimen, dose, sex, and, for the ligand binding studies, the multiple measurements made from each membrane preparation ($5HT_{1A}$ and $5HT_2$ receptors, 5HT transporter), which were then regarded as repeated measures. Since 5HT levels and turnover were assessed in cerebral cortex and hippocampus, we included the additional factor of region in the initial ANOVA. Depending upon the treatment interactions obtained in the global tests, data were then subdivided for lower order ANOVAs, followed where appropriate, by Fisher's Protected Least Significant Difference to establish effects comparing individual groups. However, in the absence of interactions of treatment with other variables, only the main effects are reported without lower-order tests. Significance was assumed at $p<0.05$. For convenience, some data are presented as the percentage change from control values; however, statistical evaluations were always carried out on the original data. For reference, control values appear in Table 1, compiled across all three control cohorts (those receiving vehicle injections on GD17-19, PN1-3, or PN7-9); however, the effects of Dex were determined only against the appropriately matched control cohort.

RESULTS

In control animals, there were only two significant sex differences among all the parameters measured (Table 1). Females displayed higher values for 5HT transporter binding in the cerebral cortex and for 5HT turnover in the hippocampus. In keeping with earlier findings with these Dex regimens (Kreider *et al*, 2005b, 2006), animals receiving doses of 0.05 or 0.2 mg/kg did not display somatic growth impairment in adulthood, whereas those given the highest dose had body weights that were about 10% below normal, with the group exposed on PN7-9 showing a statistically significant reduction ($p<0.002$, not shown). There were no significant effects on brain region weights (not shown).

Table 1 Control Values

	Cerebral cortex		Hippocampus	
Measure	**Male**	**Female**	**Male**	**Female**
$5HT_{1A}$ receptor binding (fmol/mg protein)	90±6	99±4		
$5HT_2$ receptor binding (fmol/mg protein)	101±3	99±2		
5HT transporter binding (fmol/mg protein)	367±7	393±5[a]		
5HT concentration (ng/g tissue)	268±6	271±6	217±3	221±6
5HIAA concentration (ng/g tissue)	223±14	229±12	259±18	330±25
5HT fractional turnover	0.83±0.08	0.85±0.06	1.19±0.08	1.50±0.10[a]
DA concentration (ng/g tissue)	525±25	511±19		
DOPAC concentration (ng/g tissue)	101±3	105±3		
HVA concentration (ng/g tissue)	59±2	67±3		
DA fractional turnover	0.30±0.01	0.33±0.01		

Values were compiled from all three control cohorts: those receiving saline injections on GD17-19, PN1-3, or PN7-9.
[a]Significant difference between males and females.

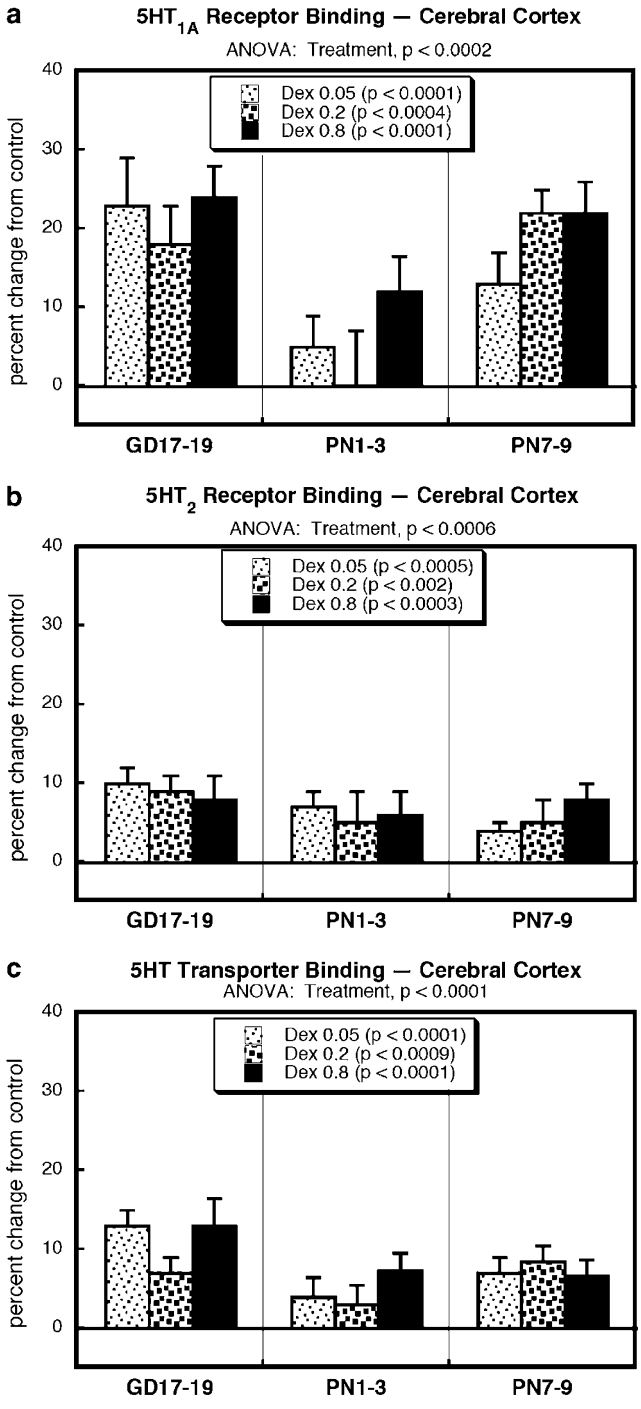

Figure 1 Effects of Dex regimens on ligand binding to $5HT_{1A}$ receptors (a) $5HT_2$ receptors (b) and the 5HT transporter (c), evaluated on PN60 and presented as the percent change from control values shown in Table 1. Across all regimens, doses, both sexes and all three measures, ANOVA identified a main treatment effect ($p<0.0001$) and an interaction of treatment × measure ($p<0.02$). Accordingly, values were separated into the three individual measures and ANOVA is shown at the top of each panel, and main effects for each dose are given within the panels. Lower-order analyses for each regimen or region were not carried out because of the absence of treatment interactions with these variables; similarly, effects on males and females were combined because of the absence of treatment × sex interactions.

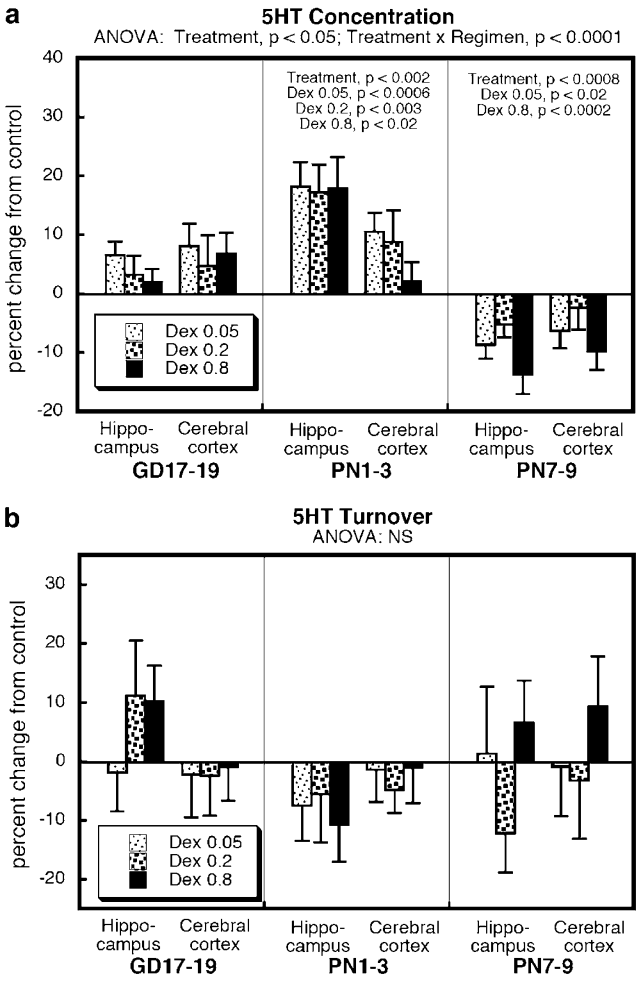

Figure 2 Effects of Dex regimens on 5HT levels (a) and fractional turnover (b), evaluated on PN60 and presented as the percent change from control values shown in Table 1. ANOVA across all regimens, doses and both sexes is shown at the top of each panel, and, because of the interaction of treatment × regimen in (a), lower-order ANOVAs for each regimen appear within the panel. Separate analyses for each region were not carried out because of the absence of treatment × region interactions and effects on males and females were combined because of the absence of treatment × sex interactions. NS, not significant.

Despite the limited effects of these Dex treatments on somatic growth, effects on 5HT and DA systems were detectable for all three regimens and even at the lowest Dex dose. For ligand-binding parameters, there were robust overall treatment effects ($p<0.0001$), with selectively greater effects on $5HT_{1A}$ receptors (treatment × measure, $p<0.02$), although significant differences were found for all three sites. Elevations of $5HT_{1A}$ receptor binding ranged as high as 25% above control values and were readily demonstrable even at 0.05 mg/kg of Dex (Figure 1a). In contrast, $5HT_2$ receptors displayed increases of no more than 10% although these, too, were statistically significant (Figure 1b). Results for the 5HT transporter resembled those for $5HT_2$ receptors, with small, but significant overall elevations at all doses across all regimens (Figure 1c). In addition, the highest dose of Dex had a somewhat greater effect on transporter binding in males than females (13 *vs* 5% elevation), which effectively eliminated the normal sex difference in this parameter.

Dex treatment also elicited increases in 5HT levels, although in this case, there were disparities among the

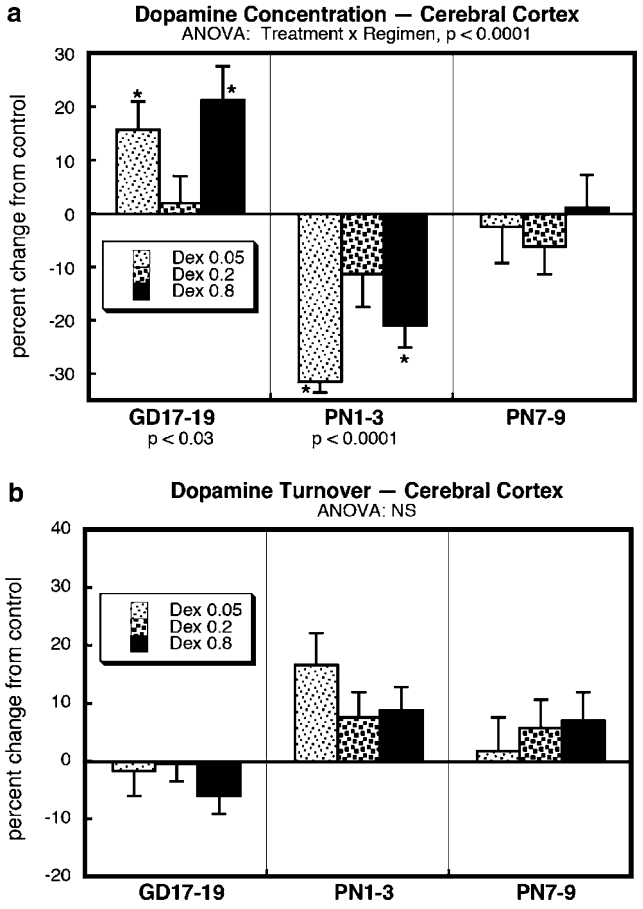

Figure 3 Effects of Dex regimens on cerebrocortical DA levels (a) and fractional turnover (b), evaluated on PN60 and presented as the percent change from control values shown in Table 1. ANOVA across all regimens, doses and both sexes is shown at the top of each panel, and, because of the interaction of treatment × regimen in (a), lower-order ANOVAs for each regimen appear below the panel; asterisks indicate individual treatment effects that differ from the corresponding control. Effects on males and females were combined because of the absence of treatment × sex interactions. NS, not significant.

three different regimens (Figure 2a). Treatment on GD17-19 did not produce significant alterations but shifting the exposure period to PN1-3 evoked increases of 10–20%, again displaying significance even at a dose of 0.05 mg/kg. When given later in the postnatal period (PN7-9), Dex instead produced significant decrements in the 5HT level. Despite the changes in 5HT levels, there were no significant differences in the fractional turnover of 5HT (Figure 2b). Furthermore, Dex treatment did not reverse the sex differences in hippocampal 5HT turnover that had been seen in the control group (Table 1): sex differences were significant for all Dex doses ($p < 0.05$ for Dex 0.05 mg/kg, $p < 0.02$ for Dex 0.2 mg/kg, $p < 0.05$ for Dex 0.8 mg/kg).

For cerebrocortical DA levels, Dex treatment had even greater effects and displayed an earlier period of vulnerability. Treatment on GD17-19 evoked elevations of up to 20% whereas the PN1-3 regimen produced even more robust decreases (Figure 3a). By PN7-9, Dex treatment no longer showed significant long-term alterations in DA levels, indicating closure of the critical period of vulnerability for this transmitter. Just as was true for 5HT, DA turnover was unaffected despite the large changes in transmitter levels (Figure 3b).

DISCUSSION

The key findings of this study are that: (1) Dex administration produces lasting alterations in indices of 5HT and DA synaptic signaling even at doses below those used therapeutically in preterm infants and in the absence of somatic growth inhibition; (2) the global upregulation of 5HT synaptic proteins occurs independently of changes in 5HT levels or turnover; and (3) the critical periods of vulnerability to Dex differ between transmitter systems even within the same brain region, implying that there is specific targeting of neurons according to their phenotype, rather than simply a generalized disruption of neural cell development. In an earlier study (Kreider *et al*, 2006), we found that deficits in cerebrocortical cell numbers were far more profound with Dex treatment on PN7-9, whereas in the current work, 5HT synaptic proteins showed a much wider window of susceptibility, with significant effects across all regimens and, if anything, slightly greater effects with GD17-19 Dex treatment. Similarly, as found earlier, effects on cholinergic synaptic activity appear to be maximal with gestational exposure rather than the PN7-9 regimen (Kreider *et al*, 2006), in keeping with the concept that the effects of Dex on specific neurotransmitter pathways is separable from those on biomarkers that reflect overall architectural features.

Indeed, the effects on 5HT levels and turnover not only reinforce this idea but also provide mechanistic insight into the effects underlying the upregulation of 5HT synaptic proteins. Unlike the global increase in the protein markers, 5HT levels showed disparate temporal effects, with no change after Dex treatment on GD17-19, an increase with the PN1-3 regimen, and a decrease after the PN7-9 exposure. In no case did we observe a change in the fractional turnover of 5HT; consequently, the changes in 5HT level are uncorrected by corresponding alterations in impulse activity and thus are likely to dictate a corresponding change in the concentration of 5HT in the synapse. Accordingly, a decrease in transmitter level without a compensatory increase in fractional turnover means a decrease in presynaptic function, whereas an increase in level without a decrease in turnover implies an increase in function. Viewed in that light, the increase in 5HT levels seen with the PN1-3 Dex treatment may be one of the reasons why the upregulation of 5HT receptors is smallest among the three regimens: increased synaptic 5HT concentrations foster receptor downregulation, partially offsetting the primary upregulation caused by Dex.

Nevertheless, it is apparent that alterations of presynaptic activity are not the sole driving force behind the effects of Dex on 5HT receptors or expression of the 5HT transporter, since upregulation was seen regardless of whether 5HT levels were unchanged (GD17-19), increased (PN1-3) or decreased (PN7-9); indeed, increases in receptors in the face of a rise in presynaptic 5HT may seem particularly puzzling, since ordinarily one would expect to see compensatory downregulation. In fact, the disconnection provides im-

portant clues as to the mechanisms underlying the effects of Dex on 5HT function. A pattern of global upregulation of synaptic proteins in the face of increased presynaptic activity has been seen with other neuroteratogens unrelated to glucocorticoids and typically reflects either a loss of postreceptor-signaling capabilities (Shahak *et al*, 2003; Yanai *et al*, 2004) or architectural 'miswiring,' where neural projections do not connect to the proper postsynaptic cells (Aldridge *et al*, 2005b; Steingart *et al*, 1998; Vatury *et al*, 2004). In either situation, the failure of synaptic signals to activate postsynaptic signaling leads to both an increase in presynaptic activity and upregulation of synaptic proteins in an unsuccessful attempt to compensate for the underlying deficiency. In the present case, there is already significant information supporting the loss of postreceptor coupling (Kreider *et al*, 2006), but certainly, miswiring may also participate in the net effects. In fact, the greater damage to architectural markers with the PN7-9 regimen (Kreider *et al*, 2006) may contribute to the decrements in 5HT levels seen with this treatment, superimposed on more direct effects of Dex on 5HT systems. This biphasic relationship, a promotional effect that is offset as neural cell loss or damage intensifies, is typical for effects of Dex on brain development (Kreider *et al*, 2005a, 2006; Slotkin *et al*, 1991; Zahalka *et al*, 1993).

Our findings for DA further support the idea of selective effects of Dex on different transmitter systems. DA levels were increased with the GD17-19 regimen but decreased after exposure on PN1-3, a temporal pattern resembling that seen with 5HT, but shifted to much earlier in development. Accordingly, the temporal transition from promotional to inhibitory effects is distinct for DA and 5HT, even within the same brain region. Furthermore, as was true for 5HT, the effects on DA levels were unaccompanied by significant changes in fractional turnover, so that the increases or decreases in levels dictate corresponding changes in DA concentrations within the synapse. This conclusion is further supported by the existence of disparate temporal patterns for effects on cholinergic and noradrenergic systems as reported previously (Kreider *et al*, 2006; Slotkin *et al*, 1992). Indeed, for the latter two transmitters, as well as for effects on cell-signaling downstream from the receptors, we found strongly sex-selective effects that served to eliminate normal sex differences between males and females (Kreider *et al*, 2005b, 2006). Although in the present study, we did find that Dex treatment eliminated the sex differences in 5HT transporter expression, it did not obtund those for transmitter turnover, so that these effects, too, appear to be transmitter selective.

The dysregulation of 5HT and DA synaptic activity in adulthood clearly contributes to the adverse behavioral outcomes noted for perinatal glucocorticoid treatments, which include persistent abnormalities of hypothalamus–pituitary–adrenal (HPA) axis function and stress responses as well as cognitive impairment (Kreider *et al*, 2005b; Matthews, 2000; Matthews *et al*, 2002; McEwen, 1992; Meaney *et al*, 1996; Welberg and Seckl, 2001). Both 5HT and DA play important roles in the setpoint for HPA reactivity (Korte *et al*, 1991; Stokes *et al*, 1987) and glucococorticoids in turn regulate the expression and function of the receptors for these two transmitters (Aghajanian *et al*, 1993; Young, 1994). It would therefore be worthwhile to investigate the extent to which early glucocorticoid exposure elicits persistent changes in HPA responses through its effects on 5HT and DA systems, or whether these animals display behavioral anomalies akin to those found in animal models of depression that involve deficient monoamine function (Jesberger and Richardson, 1985; Kelly *et al*, 1997; Song, 2000). One potential strategy is to use selective receptor antagonists to isolate the participation of specific 5HT and DA inputs in the functional outcomes (Aldridge *et al*, 2005a).

In conclusion, our results indicate that Dex administration during phases of brain development akin to those in preterm infants, produces persistent changes in indices of 5HT and DA synaptic function. Of critical importance, the effects are exerted even at doses well below those used therapeutically and are dissociated from impaired somatic growth or architectural disruption of brain development. Further, the specific involvement of cerebrocortical 5HT and DA systems means that more attention may need to be directed toward potential effects on mood, anxiety, appetitive and sleep disorders, or on reward function (Aghajanian *et al*, 1993; Nemeroff, 1998), as distinct from the past emphasis on hippocampal cholinergic systems and cognitive performance, or on hypothalamic systems and stress responses. The effects of Dex seen here strongly suggest that, within the context of its use in preterm infants (Gilstrap *et al*, 1995), adverse neurobehavioral consequences are inescapable.

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