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Corticosterone Effects on Rat Brain Template Active Region Chromatin[1,2]

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BROWN, H. AND L. UPHOUSE. *Corticosterone effects on rat brain template active region chromatin.* PHARMAC. BIOCHEM. BEHAV. 12(2) 207–212, 1980.—The effect of prenatal exposure to corticosterone on brain chromatin was examined. Pregnant Fischer inbred rats were administered corticosterone or saline on Days 17 and 18 of gestation and their offspring examined at 0 (day of birth), 2, 3, 4 or 6 days of age. *In utero* exposure to corticosterone was associated with a 24 hr delay of a developmental peak in the percentage of brain template active region chromatin. Brain and body weights of the steroid and saline-treated animals were similar, but corticosterone led to a temporary decrease in body and brain weight at 2 days of age which was reversed at 6 days of age. These results of these studies suggest an impact of corticosterone on brain gene expression.

Corticosterone Brain chromatin Template active region Development

DURING neonatal maturation, central nervous system (CNS) development is characterized by changing patterns and rates of macromolecular synthesis [7, 12, 17] which may reflect alterations in transcription of the CNS genome. Bondy and Roberts [1] reported a decline from birth to adulthood in brain chromatin template activity. Grouse *et al.* [10] showed that during this same period of development, there is an increase in the complexity of brain RNA transcripts as measured by hybridization of *in vivo* synthesized RNA to single-copy DNA. More recently, Uphouse [25] has shown that the proportion of brain chromatin in the template active region increases during early neonatal development (from birth to 3 days of age) and then declines to adult levels about 15 days of age. Several investigators have suggested that environmental manipulations may interact with these developmental profiles [11, 24, 26] but no studies have been performed with neonatal animals. In the following experiment, the effect of prenatal exposure to corticosterone on the neonatal change in the proportion of brain template active region chromatin was examined.

High pharmacological levels of glucocorticoid hormones during any period of high mitotic activity cause an almost immediate and severe depression of DNA synthesis [3]. In addition, tissue specific outcomes, independent of DNA replication [13,22], characterize the impact of glucocorticoids on target tissue. These tissue-specific influences are believed to be mediated by the effects of the steroid on RNA and protein syntheses and are presumably accompanied by altered gene expression [4]. In the brain, the possibility of altered gene regulation by glucocorticoids is evidenced by the influence of the steroid on *in vivo* RNA accumulation [3,21], but studies aimed at investigating the impact of glucocorticoids on brain transcription have not been described. In the present study, pregnant females were injected with corticosterone and their offspring examined between birth and 6 days of age. The percentage of the brain chromatin in the template active region of corticosterone and saline-treated animals was compared.

METHOD

Subjects

Forty-four nulliparous female rats of the Fischer inbred strain were purchased from Charles Rivers Laboratories, North Wilmington, MA. Upon arrival in the laboratory at 60–65 days of age, the rats were caged singly in plastic mating cages (48.3×39.4×20.3 cm or 48.3×26.7×30.5 cm) with dry pine bedding. In addition, 9 first generation females, bred in our colony, were used. These latter animals were weaned at 28 days of age and group housed in large (44.4×44.4×30.5 cm) wire mesh colony cages until they were separated into shoebox mating cages at 60–65 days of age. Both females and singly-caged stud males were maintained on a 12-12 reversed light-dark cycle (lights on: 1.00 hr) at 25.5°C and 70% humidity. All animals were allowed access to Purina rat chow and water ad lib.

Mating and Injection of Females

All interactions with the animals took place during the dark cycle. Visualization was facilitated by two red 150 W light bulbs. Purchased females were permitted one week to become familiar with colony conditions and to entrain their circadian cycles. At the end of this period, each female was tested daily for sexual receptivity by introducing a sexually experienced male into her cage. If lordosis was observed in

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response to male mounting behavior, then the male was allowed to remain with the female for 24 hr. The day of mating was counted as Day 0 of gestation. Only one female failed to conceive by this method and was subsequently discarded.

At 18.00 ± 1 hr on Days 17 and 18 of gestation, each female was removed from her cage, weighed, and injected IP with 5 mg/kg body weight of corticosterone (Sigma, St. Louis, MO) suspended in 0.9% saline, or an equivalent volume (1 ml/kg body weight) of saline vehicle alone. Beginning on the morning of Day 20 of gestation cages were checked 2–3 times a day for the presence of pups. The day a litter was found was counted as Day 0 for that litter.

Treatment of Pups

Pups were sacrificed during the middle part of the dark cycle on Day 0, 2, 3, 4, or 6 postnatally. The entire mating cage was removed from the colony to a quiet anteroom of the laboratory. Each pup was removed to a sacrifice area, weighed on a Mettler no. E200 balance steadied on a marble table, and decapitated with surgical scissors. Brains and livers were quickly removed, weighed, and frozen in liquid nitrogen. After the N_2 gas was evaporated, samples were stored in liquid scintillation vials at −80°C. Generally, an entire litter could be sacrificed in 25–35 min.

Preparation of Chromatin

Chromatin was purified by the method of Bonner *et al.* [2], as modified by Uphouse and Moore [27] but 0.1 mM phenylmethylsufonylflouride (PMSF), a protease inhibitor, was added to all solutions (except sucrose) used prior to ultracentrifugation. Whole brain or liver of an entire litter was reweighed *en masse*, and this pooled sample was used to process a single preparation of chromatin. The chromatin pellets, purified through 1.7 M sucrose (22,000 rpm, 3 hr, SW 25.1 Rotor, Beckman Model L Ultracentrifuge), were hand-homogenized into 5 ml of tris-HCl, pH 8.0 and dialyzed overnight against 266 volumes of 25 mM NaAc buffer, pH 6.6–0.1 mM PMSF.

Extraction of Template Active Region

Chromatin was fractionated as described by Gottesfeld *et al.* [8]. The dialyzed chromatin was pelleted by centrifugation at 16,000 rpm for 20 min (Beckman Model J 21 centrifuge, JA 20 Rotor). The precipitate was suspended in 25 mM NaAc buffer to a concentration of approximately 10 $A^{1}_{260}{}^{cm}$ units/ml, determined in a Varian Model 635 Spectrophotometer. Concentration of chromatin in all samples, including those fractions discussed later in this section, were determined at 260 nm in 9 volumes of 1 N NaOH and in 1 ml quartz cuvettes.

After chromatin was pre-incubated in a water bath for 5 min at 24°C, DNase II (HDAC, Worthington Biochemical, Freehold, NJ) was added in the amount of 15 units/A_{260} of chromatin. Incubation with the enzyme was continued for 20 min at 24°C with intermittent removal for vortexing. Enzymatic shearing was stopped by raising the pH to approximately 7.5 with 0.1 M tris buffer (pH unadjusted), followed by rapid cooling on ice. The chromatin was centrifuged at 16,000 rpm for 20 min to remove unsheared material (pellet-1, P1). The amount of chromatin in the supernatant (S1) was determined spectrophotometrically. Template inactive region was separated from the template active region by the dropwise addition of 1/99th volume of 0.2 M $MgCl_2$ to the S1 supernatant followed by 30 min of rapid stirring. The turbid mixture was centrifuged at 16,000 rpm for 20 min to yield a pellet-2 (P2) and a supernatant-2 (S2) fraction. The amount of chromatin in the $MgCl_2$-soluble supernatant, which contained the template active region, was determined by its absorbance at 260 nm. The percentage of chromatin in the template active region was quantified as A_{260} in S2/A_{260} in the original chromatin.

Statistical Analyses

A two-way, unweighted analysis of variance was performed on the two variables of age and prenatal treatment. Comparisons of individual means were made using a Newman-Keuls test where comparisons were within one variable [29]. The more conservative Tukey test, using the total number of cells *ab* in the place of r_{max}, was used (as suggested by Keppel [20], p.245) when a comparison was made across both variables simultaneously.

RESULTS

Offspring of 46 females were included in the analysis of growth effects. N's for brain and liver percentage template active region chromatin preparations were 42 and 36, respectively. (Due to a centrifuge failure, 4 brain chromatin samples were lost. Analysis of liver chromatin samples was discontinued when it became apparent that the developmental profile was flat over the ages examined.) Unequal n's were not the result of systematic differences, and the animals used may be assumed to be representative of the total pool.

Effects on Growth

Animals which received *in utero* exposure to exogenous corticosteroids ("treated") showed a slight but significant change in the pattern of body weight increase relative to offspring of saline-injected females ("untreated"). These data are summarized in the top of Table 1. Both main effects, age and treatment condition, were significant, $F(4,414)=428.83$, $p<0.001$ for age, and $F(1,414)=12.76$, $p<0.001$ for treatment. Post hoc Neuman-Keuls comparisons indicated that, for both groups, weight gain was so rapid that for any given day the weights were significantly higher than each of the preceding days (all p's<0.01). (For economy of space, each q value has not been presented, but they can be calculated directly from the data provided in Table 1 by using the statistics in Table 2.) Still, the pattern of weight gain was not the same for each group. Corticoid-exposed animals showed an irregular pattern which was reflected in their weighing significantly less than controls at Day 2, $q(2,\infty)=6.05$, $p<0.01$, and Day 4, $q(2,\infty)=3.13$, $p<0.05$. However, by Day 6, these animals actually weighed more than controls, but not significantly so, $q(2,\infty)=1$. The reversal of ordinal position was reflected in a significant interaction between condition and age, $F(4,414)=3.27$, $p<0.05$.

Corticosterone treatment resulted in brain deficits that resembled those in body. The main effects for age, $F(4,417)=395.43$, $p<0.001$ and for treatment, $F(1,417)=3.80$, $p<0.05$ were both statistically reliable, as was the interaction, $F(4,417)=5.19$, $p<0.001$. Brain weight mimicked body weight in that the former also demonstrated such pronounced agewise increases that weight was consistent-

TABLE 1

BODY, BRAIN AND LIVER GROWTH IN NEONATES EXPOSED TO PRENATAL TREATMENTS

Treatment[a]	Age 0	2	3	4	6
Body					
Sal	5.19 ± 0.42 (33)	6.73 ± 0.47 (46)	7.41 ± 0.61 (40)	8.11 ± 1.02 (41)	9.25 ± 1.05 (48)
Cort	5.07 ± 0.49 (44)	6.08 ± 0.56 (48)	7.21 ± 0.81 (29)	7.77 ± 0.59 (39)	9.34 ± 1.05 (56)
% dif§	−2.3	− 9.66†	−2.7	− 4.2*	+1.0
Brain					
Sal	231 ± 34 (33)	312 ± 48 (46)	351 ± 41 (40)	403 ± 79 (42)	492 ± 34 (50)
Cort	224 ± 34 (44)	275 ± 37 (48)	339 ± 47 (29)	384 ± 38 (39)	521 ± 59 (56)
% dif	−3.2	−11.7†	−3.4	− 4.9	+5.9†
Liver					
Sal	224 ± 38 (33)	233 ± 33 (46)	237 ± 51 (40)	241 ± 43 (41)	235 ± 30 (49)
Cort	222 ± 40 (44)	216 ± 50 (48)	249 ± 35 (28)	208 ± 47 (39)	243 ± 47 (55)
% dif	−0.7	− 7.5	+5.2	−13.8†	+3.6

*$p<0.05$; †$p<0.01$; ‡Sal, saline; Cort, corticosterone; §% dif=(Cort−Sal)/Sal×100.

Mean body weight is expressed in grams ± SD. Mean organ weight is expressed in milligrams ± SD. n's are in parentheses. Mothers of these animals received either 5 mg/kg body weight of corticosterone or an equivalent volume of saline on Days 17 and 18 of gestation.

TABLE 2

STATISTICS FOR CALCULATIONS OF NEWMAN-KEUL RATIOS FOR GROWTH RATES

	MS_e	df_e	N*
Body	0.4715	414	41.016
Brain	0.0023	417	41.255
Liver	0.0018	413	40.827

The following formula may be used to obtain the calculated Studentized Range statistic for any pair of ranked means within any single level of a factor:

$$q = \frac{\bar{X}_1 - \bar{X}_2}{(MS_e/n)^{.5}}.$$

*harmonic mean.

ly statistically greater on Day n than on Day n−1 (all p's<0.05). At Day 0 weights between the groups were not different, but by Day 2, corticoid-treated pups exhibited brain weights that reached only 88% of that of controls, $q(2,\infty)=4.86$, $p<0.01$, see Table 1. From this age, brain weight gain in the saline group remained almost perfectly linear, as it had been from birth. Interestingly, corticoid pups began to show a progressive recovery from the deficit at two days such that their brain weights were not significantly below controls at Days 3 and 4, and were significantly *larger* at 6 days of age, $q(2,\infty)=3.89$, $p<0.01$.

Liver revealed characteristics of both the general body weight and brain weight trends, while exhibiting patterns unlike either. Liver weight increased with age, $F(4,413)=4.12$, $p<0.01$, but there were no significant overall differences between hormone and saline-treated animals. However, there was a significant age by treatment interaction, $F(4,413)=4.06$, $p<0.01$. This interaction seemed to result from a significant decrease in the weight of livers of corticoid treated pups at Day 4, $q(2,\infty)=4.99$, $p<0.01$, interdigitated with liver values that were higher than controls at Days 3 and 6 (see Table 1).

By way of summary, in both brain and body of controls, a practically linear increase in weight was seen during the 0–6 day period studied. A plot of liver weights from controls would also yield a straight line, but one that rises less across ages. Corticosterone-exposed animals demonstrated, in body and liver, a characteristic fall from control levels between birth and 2 days, recovery at 3 days, and a decrease again at 4 days of age. In brain, corticoid-treated animals showed no such phasic recovery, and tended to remain tonically depressed below levels of untreated animals between 0–4 days of age. In all three measures control values were lower at Day 6, but only in brain was this difference significant. The overall similarity of the curves can be seen in the "% dif" row of Table 1. A summary of statistics which can be used to calculate any mean comparisons not explicitly given in the text are presented in Table 2.

Effects on Template Active Region Chromatin

The data for brain percentage template active region chromatin are shown in Fig. 1. As can be seen, template active region chromatin from both treated and untreated pups peaks during early neonatal development. Analysis of variance revealed a significant effect of age, $F(4,32)=5.60$, $p<0.01$. However, the percentage of chromatin in the template active fraction peaks in one group while there is a trough in the other group. This served to cause mutual cancellation of between-group differences, $F(1,32)<1$. Because of the infidelity of the two different peaks, a significant interaction emerged, $F(4,32)=4.08$, $p<0.01$. In the corticosterone-treated group, post hoc comparisons revealed that 4 day old animals were different from animals at 0, 3, and 6 days, but not from the 2 day group. The q values that led to these conclusions are listed, and represented in graphic form, in Table 3(a).

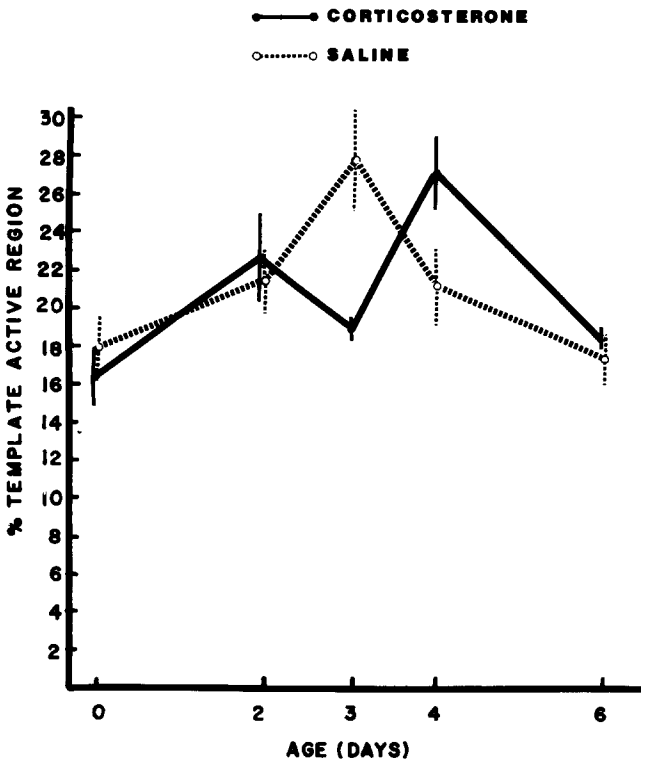

FIG. 1. The effect of maternal corticosterone treatment on the proportion of whole brain template active region chromatin of offspring. (Values are expressed as the mean ± the standard error. n's per age=4, except in the 6 day groups, in which n=5.)

Within the saline group, the rise in percentage template active region chromatin seen at 3 days is significantly different from the other four ages studied, which do not differ among themselves (see Table 3, section b). Within ages 0, 2, and 6 day, corticosterone and saline-treated animals did not differ in their average percentage template active region. Both the 3 and 4 day comparisons differed significantly (see Table 3, section c). Analysis of the 3 day peak in saline animals versus the 4 day peak in corticoid animals was done by use of the Tukey test. This procedure yielded a mean critical range. $\overline{CR}_T$, which was equal to 9.0. This value was much larger than the mean difference between the 3 and 4 day peaks, 0.6; hence, these peaks are shifted in time, but not in magnitude.

In contrast to the effects of corticosterone on brain chromatin template active region, in liver, no differences were found in the proportion of chromatin fractionating into the template active region (see Fig. 2).

TABLE 3

SUMMARY OF NEUMAN-KEULS FOR BRAIN TEMPLATE ACTIVE REGION CHROMATIN

corticoid						
(a)	age	0	6	3	2	4
	Mean	16.44	18.39	18.91	22.58	27.10
		4 vs 0 $q(5,30)=5.60$, $p<0.01$				
		4 vs 6 $q(4,30)=4.58$, $p<0.05$				
		4 vs 3 $q(3,30)=4.31$, $p<0.05$				
		4 vs 2 $q(2,30)=2.38$, $p>0.05$				
saline						
(b)	age	6	0	4	2	3
	mean	17.23	17.90	21.08	21.30	27.71
		3 vs 6 $q(5,30)=5.51$, $p<0.01$				
		3 vs 0 $q(4,30)=5.16$, $p<0.01$				
		3 vs 4 $q(3,30)=3.49$, $p<0.05$				
		3 vs 2 $q(2,30)=3.37$, $p<0.05$				
across treatment						
(c)	age	0 vs 0 $q(2.30)$ 1				
		2 vs 2 $q(2,30)$ 1				
		3 vs 3 $q(2,30)=4.62$, $p<0.01$				
		4 vs 4 $q(2,30)=3.17$, $p<0.05$				
		6 vs 6 $q(2,30)$ 1				

DISCUSSION

Effects on Growth

Injection of corticosterone on Days 17 and 18 of pregnancy caused significant decreases in brain and body weights of offspring, although the size and persistence of these differences were much less than those reported by others [5, 6, 13, 14, 16, 17]. Body weight of animals exposed to prenatal corticoids, which was 12% lower than controls at its maximum, is markedly different from the 25–43% reductions reported previously (e.g., see Howard and colleagues

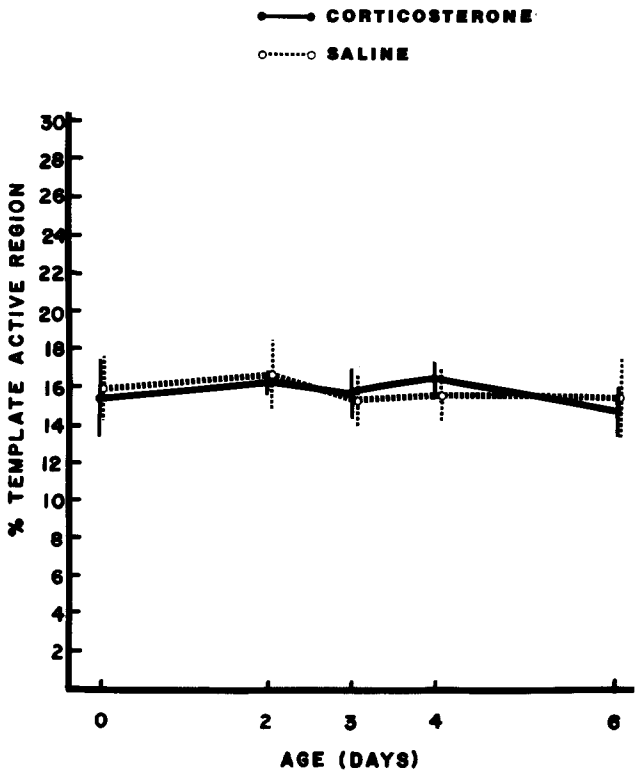

FIG. 2. The effect of *in utero* corticosterone exposure on liver percentage template active region chromatin. (Values are expressed as the mean ± the standard error. n's per age=4, except in the 4 and 6 day groups, in which n=3.)

papers). Further, in the present report, body weight completely recovered and actually exceeded control values at Day 6. While previous studies have found animals treated neonatally to evidence improvement, these animals typically did not recover absolute weight values. Perhaps the recovery found in the present experiment represents the rebound period that follows treatment, and the smaller magnitude of effects allowed greater recovery to occur. Still any of a number of methodological differences (e.g., drug dosage, time after injection, strain of animals) between past and present studies might account for the disparities.

Effects on Template Active Region Chromatin

Knowledge of the normative developmental patterns of the rodent brain [7, 9, 30] and the effects of glucocorticoids on these patterns suggest a promising avenue for the study of the underlying mechanisms responsible for the influence of the steroid on CNS development. Previous studies [25] demonstrated that the percentage of brain template active region chromatin increased from birth to 3 days of age after which it declined to adult levels at 15 days of age. In the present study, this neonatal increase and decrease in the proportion of brain template active region chromatin was replicated in the saline-treated animals, but the increase was delayed by 1 day in the corticosterone treated offspring. Both corticosterone and saline-treated animals exhibited similar developmental profiles, even reaching similar peak changes in the percentage of template active region chromatin; the only difference between the two groups appeared to be the time at which the peak occurred.

These developmental profiles, while similar to those reported by Uphouse [25] are not identical. Uphouse demonstrated an apparent plateau in the percentage of brain template active region chromatin which spanned the ages of 3–6 days of age. In the present study, both corticosterone and saline-treated offspring returned to baseline 1 day after the neonatal peak. With the present data, we cannot definitively address the differences between the two studies. However, it is important to note that in the previous Uphouse study, pregnant females were neither handled nor injected, and the females were not nulliparous. These differences may have contributed to the rate at which the postnatal change in percentage of template active region chromatin occurred.

In contrast to the effect of prenatal exposure to corticoterone on brain chromatin, no differences were seen between saline and hormone-treated offspring in the percentage of liver chromatin fractionating as template active region chromatin. This is surprising in view of the effect of hydrocortisone on liver chromatin template activity [4]. However, the lack of an influence by the steroid on liver is compatible with the observation by Uphouse [25] that the developmental profile for proportion of liver template active region chromatin is essentially flat from birth to adulthood. These data cannot rule out an influence of the prenatal treatment on liver chromatin for significant changes may have occurred *in utero* which would have escaped the present postnatal analysis.

Different findings for the effects of corticosterone on liver chromatin template activity and the chromatin template active region are not necessarily contradictory. The measurement provided similar (but not identical) information about the transcriptional activity of the chromatin. Template activity provides an estimate of the percentage of the chromatin undergoing transcription. However, the template active region chromatin is believed to include, in addition to these actively transcribed sequences, sequences that are potentially transcribable [28]. The sequences transcribed in an assay for chromatin template activity probably represent only a subset of those sequences extracted as the template active region.

The postnatal effect of prenatal corticosterone treatment on brain chromatin is interesting for several reasons: (1) it demonstrates that the developmental change in the percentage of brain template active region chromatin can be modulated by events occurring at previous intervals; (2) it demonstrates that the effects of glucocorticoids on brain chromatin are not restricted to the period of heightened plasma levels; and (3) glucocorticoid modulation of brain template active region chromatin may provide insight into the functional significance of greater levels of transcriptional activity in neural tissue during neonatal development. Since in the present study, only the template active region was measured, it is not known if the one-day delay in the brain chromatin developmental profile is also reflected in a delay in the transcription of the brain genome. However, glucorticoids are believed to influence brain development (e.g., gliogenesis, synaptogenesis and myelination) and a change in transcriptional activity may be necessary for one or all of these challenges on metabolic rate.

Glucocorticoids have been reported to influence behavioral development [15, 16, 18, 23] and in many cases the development is delayed in a fashion similar to that observed with the brain template active region chromatin. It is not unreasonable to suppose that the delay in the brain template active region chromatin peak results in a disruption of behavioral development. Functional maturation which is dependent upon CNS organizational events occurring at 3–4 days of age might be delayed, or even eliminated from appearing. However, whether the delay in brain chromatin change produces or is a consequence of a glucocorticoid interruption of CNS development is unknown. Consequently, whether the template active region peak delay is causally related to or only correlated with behavioral change remains to be determined.

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