

PERINATAL PROGESTERONE AFFECTS LEARNING IN RATS

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SUMMARY

(1) There has been considerable discussion in recent years concerning reported effects of prenatally administered progesterone or synthetic progestins on the intelligence and personalities of children. (2) Very little work has been done with animals, in which physiological mechanisms may be sought. (3) We report here that pre- and early postnatally administered progesterone facilitated rats' performance of an active avoidance task both prepubertally and in young adulthood. (4) This, together with other recent results from our lab, suggests a demasculinization of behavior patterns, perhaps mediated by alterations of monoamine systems.

Key Words—Progesterone; perinatal hormones; active avoidance; learning; rats.

INTRODUCTION

PERINATAL exposure to altered levels of gonadal hormones has repeatedly been shown to affect later reproductive behavior of male and female rats, as well as a number of non-reproductive behaviors. (See Archer, 1975; Goldman, 1978; Ward, 1974, for reviews.) Typically, exposure of neonatal (or in some cases prenatal) females to testosterone has been reported to facilitate behavior patterns in adulthood which are more frequently exhibited by males (copulatory mounting, decreased activity levels, increased aggressiveness, better maze performance, slower active avoidance behavior and increased defecation in an open field apparatus). A shift in the opposite direction occurs in adult males castrated in infancy and/or exposed to antiandrogen pre- or neonatally. However, few studies have examined the effects of perinatal progesterone on later behavior. There have been suggestions that progesterone administered to the mother during pregnancy increases IQ and/or academic performance of children (Dalton, 1976; Ehrhardt & Money, 1967). However, concerns about the adequacy of control groups (Meyer-Bahlburg & Ehrhardt, 1977; Quadagno, Brisco & Quadagno, 1977; Reinisch, 1976), as well as negative results in well controlled studies (Meyer-Bahlburg, Grisanti & Ehrhardt, 1977; Ehrhardt, Grisanti & Meyer-Bahlburg, 1977; Reinisch, 1977) have brought these findings into question. The issue has not been resolved, however, because the negative results were obtained with lower doses and with synthetic progestins, rather than with the higher doses of natural progesterone used in Dalton's studies.

Coyle, Anker & Cragg (1977) reported differences on only three of 29 measures of neonatal

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physical, behavioral and biochemical parameters, subsequent to prenatal progesterone administration to rats. They concluded that progesterone in the dose they used exerts no consistent effect on early development of rats. On the other hand, Stevens & Goldstein (1978) reported a complicated pattern of effects of single neonatal injections of progesterone and testosterone on open field behavior of adult male and female rats. The authors interpreted some of the results of the progesterone administration as indicative of a shift towards behavior typical of females. We have recently provided evidence for various behavioral and biochemical changes in rats associated with perinatal exposure to progesterone. Males administered a relatively high dose of progesterone both pre- and postnatally via maternal implants exhibited impaired adult performance in a Lashley maze, while females' performance was unaffected by the progesterone treatment (Hull, Franz, Snyder & Nishita, in press). These animals showed a nonsignificant trend towards improved performance on an active avoidance task. Males whose dams received only postparturitional injections of progesterone exhibited significantly fewer and longer latency ejaculations and consistent trends toward reductions in other copulatory and aggressive behaviors. These results are consistent with the hypothesis of demasculinization of behavior patterns. No significant differences were found in active avoidance behavior of these animals.

The present study was designed to analyze further the effect of perinatal progesterone on behavior in infancy and adulthood. Progesterone was administered daily to the mothers prior to and for the first 3 weeks after parturition. Doses injected in this experiment were higher than the amounts expected to diffuse daily from the implants in the first experiment above (Hull *et al.*, in press) and extended for a longer time (including the pre- as well as postnatal periods) than in the second experiment. Half the animals in this experiment were tested on an active avoidance task in infancy, the other half, in adulthood. In addition, running wheel activity and body weights were measured.

METHODS

Subjects

The subjects were 87 offspring of 15 Long-Evans female rats, which were time mated and housed individually throughout gestation and with the litter during lactation. Each dam received injections of progesterone or vehicle alone as described below. Food pellets and water were available *ad lib.* throughout the experiment. Half the pups in each litter were tested at 19 days of age on a one-way active avoidance task. Animals not trained as infants were tested in early adulthood (65-75 days of age) on a similar task.

Apparatus

Infant active avoidance tests were conducted in a Lehigh Valley shuttlebox, 26 × 9.5 × 13 cm. Grid bars were 6 mm apart, center to center. A 13 × 9.5 cm piece of Plexiglas covered one end of the grid floor. Adults were trained in a similar but larger Lehigh Valley shuttlebox (46 × 20.5 × 20 cm). A 4 cm high barrier divided the shock compartment from the safe one. Grid bars were 11 mm apart. Activity levels were measured in Wahman automated running wheels.

Procedure

Subcutaneous injections of 3.3 mg/kg progesterone or an equivalent volume of the olive oil vehicle were begun on day 8 of gestation. Daily injections continued through day 18, at which time they were discontinued for 5 days to allow for normal deliveries. Litters were culled to 9-12 at birth and allowed to remain with their dams. Maternal injections were resumed on the day after parturition (postnatal day 2) and continued for 21 days, each animal receiving the same treatment as during gestation.

Half the offspring in each litter were tested in the small shuttlebox beginning at 19 days of age. If the animal failed to run to the safe end within 7 sec of being placed into the apparatus, a 0.2 mA footshock was administered. Maximum shock duration was 53 sec. Failure to run within the 60 sec trial resulted in the

animal's being gently nudged onto the platform. Each animal was given 15 trials separated by 20 sec rests in a holding cage. The following day 15 trials were again administered, the first 5 with shock (retention) and the last 10 without shock (extinction). The latency to escape or avoid was recorded for each trial, and the data analyzed in blocks of 5 trials. The percentage of trials in which an avoidance (crossing within 7 sec) was made was calculated for each animal for its 15 acquisition and 10 extinction trials. Statistical analyses were performed using a 3-way analysis of variance (Winer, 1971), with gender, treatment and blocks of trials as factors. Animals not trained in active avoidance as infants were tested in early adulthood (65–75 days) in the large shuttlebox. The procedure was the same as with the infants, except that a 0.4 mA shock was administered.

Activity levels were determined at 60–70 days of age, prior to active avoidance testing. Animals were allowed to adapt to the wheels for 1 hr/day for 3 days. Vaginal smears were taken from females during this time and immediately prior to testing, in order to determine the stage of the estrous cycle. After adaptation, animals were left in the wheels for 24 hr with food pellets and water available in the adjacent cage. Scores were recorded at two 12-hr intervals: 12 hr in light (09:00–21:00 hr), then 12 hr in dark (21:00–09:00 hr). Scores were analyzed with a 2-way analysis of variance, with gender (including males, estrous females and diestrous females) and treatment as variables.

RESULTS

The results of the infant active avoidance testing are illustrated in Fig. 1. Progesterone treated pups exhibited faster latencies in performance of the entire task [$F(1,190) = 5.18$, $p < 0.03$]. This is reflected both in improved acquisition of the response [$F(1,88) = 8.09$,

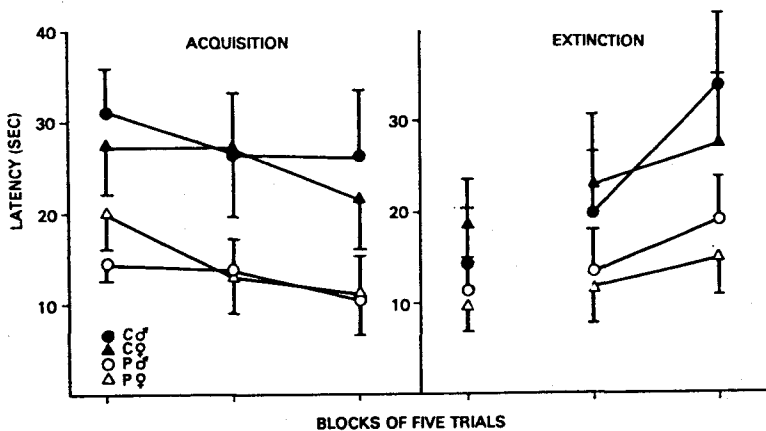


FIG. 1. Mean latencies for infant active avoidance task. P group had faster latencies on acquisition ($p < 0.01$) and on extinction ($p < 0.05$).

$p < 0.01$] and in faster latencies in extinction, i.e. a greater resistance to extinction [$F(1,76) = 9.34$, $p < 0.005$]. As seen in Table I, progesterone treated animals made significantly more avoidances during the acquisition phase [$F(1,44) = 9.91$, $p < 0.01$]. No gender differences were obtained. Adult active avoidance data are presented in Fig. 2. Rats receiving perinatal progesterone showed enhanced active avoidance performance during young adulthood compared with controls [$F(1,160) = 4.97$, $p < 0.05$]. These animals exhibited shorter latencies to respond during acquisition [$F(1,64) = 5.42$, $p < 0.05$], but no significant increase in percent avoidance, as seen in Table I. During extinction a trials \times progesterone interaction was obtained for latency data [$F(1,32) = 8.51$, $p < 0.01$] which reflects later

TABLE I. MEAN PER CENT AVOIDANCE OF ALL RATS

Group	Acquisition	Extinction
ICM	21.8 ± 7.3 (11)	28.8 ± 10.9 (8)
ICF	23.0 ± 7.6 (11)	44.4 ± 14.8 (9)
IPM	35.2 ± 6.5 (14)	48.6 ± 9.2 (14)
IPF	43.9 ± 5.4 (12)	51.8 ± 11.1 (11)
ACM	13.3 ± 8.2 (5)	14.0 ± 14.0 (5)
ACF	35.2 ± 9.4 (11)	16.4 ± 8.2 (11)
APM	36.1 ± 8.3 (11)	31.8 ± 10.9 (11)
APF	50.4 ± 10.7 (9)	41.1 ± 14.6 (9)

Group means ± S.E. of per cent avoidances, calculated as percentage of 15 trials during acquisition and 10 trials during extinction for each animal. Number of animals per group given in parentheses. ANOVA revealed a greater percentage of avoidances by progesterone treated infants during acquisition ($p < 0.01$). (ICM—infant control males, ICF—infant control females, IPM—infant progesterone males, IPF—infant progesterone females, ACM—adult control males, etc.).

Animals which consistently failed to escape during acquisition training were not tested for extinction.

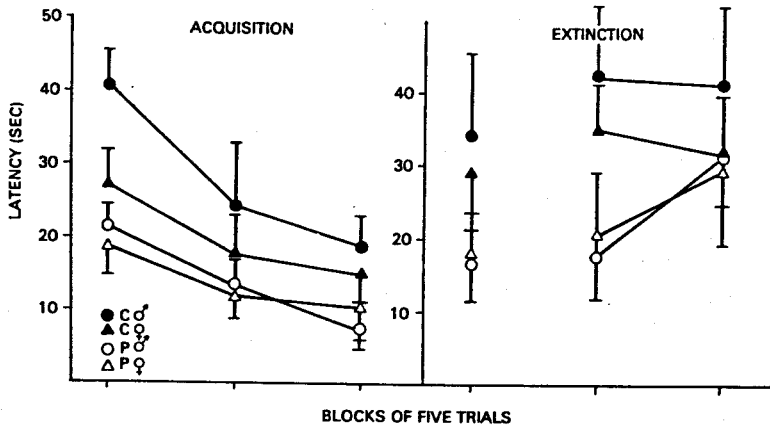


FIG. 2. Mean latencies for adult active avoidance task. P group had faster latencies during acquisition ($p < 0.05$). A trials × progesterone interaction in extinction ($p < 0.05$) reflected delayed extinction.

TABLE II. MEAN RUNNING WHEEL ACTIVITY SCORES

Males	Estrous females	Diestrus females
C 896.2 ± 142.5 (5)	2517.8 ± 380.8 (5)	1317.8 ± 296.6 (6)
P 708.9 ± 102.6 (11)	2000.8 ± 625.1 (4)	1272.2 ± 199.0 (5)

Group means ± S.E. of 24-hr running wheel activity scores. ANOVA revealed activity differences as follows: estrous females > diestrus females > males, $p < 0.001$.

extinction by progesterone treated animals than controls. Trends toward better performance by adult females than adult males in both latency and per cent avoidance did not attain statistical significance.

In order to determine whether differences in active avoidance responding may be due to differences in basal activity levels, we measured 24-hr running wheel activity. These data are shown in Table II. A significant gender difference was obtained, estrous females being more active than diestrous females, and males showing the least activity [$F(2,30) = 14.17$, $p < 0.001$]. No significant differences due to progesterone were obtained, control animals showing somewhat greater activity. Thus, the improved active avoidance performance is not due to greater activity on the part of progesterone treated animals. To determine whether progesterone treatment had any effect on body weight in these animals, we recorded body weights prior to adult active avoidance testing. Weights of progesterone treated males (mean = 359.5, S.E. = 11.3) and females (218.1 ± 6.5) were not significantly different from those of control males (371.6 ± 14.4) and females (225.5 ± 3.1).

DISCUSSION

The results of this study indicate that relatively long term perinatal progesterone treatment can improve acquisition and retard extinction of an active avoidance response by prepubertal and young adult animals. Since female rats are generally better at active avoidance tasks (Archer, 1975), this improved performance subsequent to perinatal progesterone treatment may represent a demasculinization or feminization of behavior. Neonatal hormonal treatment has been shown to alter the normal gender dimorphic patterns on this task. Female rats treated with testosterone in infancy responded like males in a two-way active avoidance task, and were significantly worse than untreated females (Beatty & Beatty, 1970). While early castration of males did not improve their active avoidance performance as adults, neonatal castration following prenatal treatment with cyproterone acetate (an antiandrogen) did feminize their responses as adults so that their performance was indistinguishable from that of normal females (Scouten, Grotelueschen & Beatty, 1975). Previous studies in our laboratory have demonstrated that male rats exposed to progesterone perinatally exhibited less effective copulatory behavior, longer latencies to the first aggressive encounter, and poorer maze performance (Hull *et al.*, in press), all of which have been considered to be less masculine and/or more feminine patterns of behavior. Most gender differences in behavior are not manifested until puberty. Since progesterone's facilitation of avoidance behavior was observed both before and after puberty in this experiment, the increased levels of endogenous steroids associated with puberty were not a factor in this effect. Furthermore, the progesterone effect was considerably greater than the nonsignificant trend for adult control females to have faster latencies than males.

Previously we found that neither high doses of progesterone administered postnatally nor much lower doses administered both pre- and postnatally were sufficient to interfere with "masculinization" of avoidance responding (Hull *et al.*, in press). In combination, these studies suggest that either the period of maximal sensitivity for this effect occurs prenatally, or prolonged exposure to greatly elevated progesterone levels is necessary. Maisel, Dohanich & Ward (1979) found that prenatal stress was sufficient to facilitate lordosis responding in males, but not sufficient to feminize active avoidance performance.

This is consistent with the hypothesis that prolonged interference with androgens during perinatal development is necessary to facilitate active avoidance performance. We have demonstrated that our procedure significantly elevated neonatal progesterone levels. Using an injection regimen almost identical to the present one, we found progesterone levels in 7-day-old pups to be 240% those of controls (3.99 ± 0.52 ng/ml for progesterone treated animals, 1.62 ± 0.14 ng/ml for controls; Franz, Hull, Snyder & Roth, 1978).

The mechanism(s) mediating the effects of perinatal progesterone have not been specified. It has been suggested that progesterone antagonizes the masculinizing effects of the high levels of endogenous androgens and estrogens present during the first few days after birth in rats and prenatally in primates (Resko, 1975; Shapiro, Goldman, Bongiovanni & Marino, 1976). However, the specific molecular mechanisms of this action are unknown. Brain monoamines have been implicated in the control of steroid hormone secretion (Anton-Tay & Wurtman, 1971). Thus, progesterone may exert its developmental influence via alterations in brain monoamine levels. We have demonstrated increased monoamine oxidase (MAO) activity in the brains of 7-day-old progesterone treated pups (Franz *et al.*, 1978) and in day 20 fetuses (Snyder, Hull & Roth, 1979). Furthermore, male sexual behaviors and performance of an active avoidance task were permanently altered by neonatal administration of drugs which affect monoamine levels (Dorner, Hecht & Hinz, 1976).

The present results are especially interesting in light of studies demonstrating that high levels of progesterone during adulthood impaired females' acquisition (Bannerjee, 1971) and facilitated males' and females' extinction (van Wimersma Griedanus, Wijnen & de Weid, 1973) of active avoidance responses. Progesterone is known to have mildly sedative and anesthetic effects (Aufreere & Benson, 1976), and while activity levels were unchanged in the van Wimersma Griedanus study, reactivity to shock may have been diminished. Whether or not this is the correct explanation of the difference, it is clear that perinatally administered progesterone influences adult active avoidance performance in a manner opposite to that of high levels of progesterone present during the task. Reinisch (1976, 1977) has suggested that the effects of prenatal progesterone on school achievement reported by Dalton (1976) are more likely to be due to general behavioral dispositions than to increases in intelligence. While perinatal progesterone clearly facilitated active avoidance behavior in rats in the present experiment, it also impaired maze performance and sexual and aggressive behaviors in a previous experiment in our laboratory (Hull *et al.*, in press). Thus, its effects on rats also appear to be more varied than a simple facilitation of learning. Whether all of these effects can be related to gender dimorphism remains to be demonstrated.

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