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Hormonal Mechanisms Underlying Aberrant Sexual Differentiation in Male Rats Prenatally Exposed to Alcohol, Stress, or Both

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The male offspring of rats exposed to restraint stress, alcohol, or both during late pregnancy show normally masculinized genitalia; however, sexual differentiation of behavior is dissociated from the external morphology. In contrast to controls, males exposed prenatally to stress, alcohol, or a combination of these factors exhibited the female lordotic pattern. Thus, all 3 prenatal treatments led to incomplete behavioral defeminization. Behavioral masculinization was not altered by fetal alcohol exposure alone, but a significant number of males that experienced prenatal stress alone failed to copulate. A more severe disruption of behavioral masculinization occurred when stress and alcohol were combined. Very few males exposed to the combination treatment mated with females. This study attempted to relate the effects of these treatments on sexual behavior to the postparturitional surge in plasma testosterone (T) that is known to influence the process of sexual differentiation. Prenatally stressed males, like control males showed a large, brief surge in plasma T that peaked 1 hr after delivery. Altered defeminization and masculinization were seen in prenatally stressed males, despite a normal postparturitional T surge. Fetal alcohol exposure, with or without concomitant stress, depressed T to the same extent right after birth and led to a similarly blunted T surge 1 hr later. Thus, equal disruption of the neonatal T pattern occurred in alcohol-alone males, who showed normal male copulatory behavior, and in alcohol-plus-stress males, whose behavior was severely attenuated. The results suggest that consideration of abnormal exposure to T during prenatal ontogeny may be required to understand the atypical sexual behaviors associated with these treatments.

KEY WORDS: prenatal stress; fetal alcohol; sexual behavior; male rats; postparturitional testosterone surge.

INTRODUCTION

A compelling biological principle underlying the differentiation of sexual behaviors is the axiom that mammals have a basic tendency to develop along feminine lines. What prevents expression of the default female traits is exposure to testosterone (T) or one of its metabolites during perinatal life. Normally, the gonads of males release appreciable quantities of androgen during this period, leading to masculinization and defeminization of reproductive behaviors, and sexually dimorphic anatomical structures, including the nervous system. Females normally develop in a hormonal milieu low in androgenic steroids, ensuring that the predisposition for dimorphic tissue to be feminized is retained. This general scenario seems to hold for most mammalian species (for review, see Ward & Ward, 1985), most likely including humans (LeVay, 1993).

Sexual differentiation is vulnerable to any factors capable of causing appreciable deviations from the normal pattern of androgen release by the perinatal testes. In previous studies, we have evaluated the consequences of exposing pregnant rats to two such factors on the sexual behaviors of their male offspring. Prenatal alcohol and

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stress were selected because of their potential relevance to the human condition.

Prenatal exposure of male rats either to ethanol or to stress alters the differentiation of sexual behavior potentials. Males prenatally subjected to stress (Ward, 1972a, 1977; Ward & Reed, 1985) or to alcohol (Broida et al., as cited in Ward, 1992; Hård et al., 1984; Ward, Ward, Winn, & Bielawski, 1994) alone, or in combination (Ward et al., 1994), exhibit a markedly enhanced capacity to display lordosis, the female sexual behavior pattern. Although an atypical display of female behavior is a consistent finding, effects of these treatments on the male sexual pattern are more variable. Most studies evaluating male copulatory behavior in prenatally stressed rats found a significant reduction in the number of males that initiate copulation (e.g., Dunlap, Zadina, & Gougis, 1978; Ward, 1972a, 1977; Ward & Reed, 1985; Ward, Bennett, Ward, Hendricks & French, 1999); however, in some studies, prenatally stressed males ejaculated normally (e.g., Dahlöf, Hård, & Larsson, 1977; Ward, Monaghan, & Ward, 1986; Ward et al., 1994; I. L. Ward et al., 1996). Fetal exposure to alcohol alone is usually reported to have little effect on male copulatory behavior in rats (Chen & Smith, 1979; Dahlgren et al., 1989; Hård et al., 1984; I. L. Ward et al., 1996; Ward et al., 1999), although minor decrements are occasionally noted (McGivern, Handa, & Raum, 1998; Ward et al., 1994). However, when prenatal exposure to stress and alcohol are combined, there is a profound and highly reliable deficit in the ability of males to ejaculate (Ward et al., 1994, 1999; I. L. Ward et al., 1996). Less than one quarter of these males initiated copulation when given prolonged access to estrous females (Ward et al., 1999; I. L. Ward et al., 1996).

The atypical sexual behaviors of males derived from dams exposed to stress, ethanol, or both during pregnancy suggest that the treatments interfere with the normal pattern of plasma T prevailing during the specific perinatal stages when sexual differentiation is ongoing. In the rat, males normally experience elevated levels of plasma T during Days 18 and 19 of gestation (Ward & Weisz, 1984; Weisz & Ward, 1980). A second, briefer, but larger surge in plasma T occurs during the first few hours after birth (Baum, Brand, Ooms, Vreeburg, & Slob, 1988; Corbier, Kerdelhue, Picon, & Roffi, 1978; Lalau, Aubert, Carmignac, Gregoire, & Dupouy, 1990; Slob, Ooms, & Vreeburg, 1980). Each of the two androgen spikes has been shown to contribute to the process by which adult sexual behavior potentials are masculinized and defeminized in rats. For example, females exposed prenatally to a single dose of testosterone propionate (TP) or free T on Days 18 or 19 of gestation showed reduced receptivity in adulthood compared to females exposed to T earlier in gestation (Huffman & Hendricks, 1981; Nadler, 1969; Rhees, Kirk, Sephton, & Lephart, 1997). Furthermore, TP given on Day 18 increased masculine copulatory tendencies in females (Nadler, 1969).

Defeminization and masculinization of sexual behavior potentials also are mediated by T exposure during neonatal life. Female rats injected with a low dose (5 μg) of TP 1 hr after birth exhibited a greater suppression in lordosis than females injected 24 hr later (Thomas, Howard, & Barfield, 1983). Similarly, male rats spayed within 1 hr of birth showed both higher levels of lordosis (Corbier, Roffi, & Rhoda, 1983; Thomas & Gerall, 1969) and less mounting of females (Roffi, Chami, Corbier, & Edwards, 1987) than those gonadectomized at 6 hr. Likewise, the normal differentiation of dimorphic CNS structures is influenced by exposure to the two perinatal surges in T (Rhees, Shryne, & Gorski, 1990; O. B. Ward, Wexler, Carlucci, Eckert, & Ward, 1996).

Prenatally stressed or alcoholized males (or both) all have a similarly augmented potential to display the female lordotic pattern, suggesting that the treatments induce a shared alteration in the perinatal T milieu. On the other hand, the male copulatory pattern of animals exposed to a combination of stress and alcohol is more reliably and severely attenuated than in males that had experienced only one treatment. Thus, stress brought together with alcohol may cause a particularly severe or temporally unique flaw or both in early T.

Abnormalities in fetal T on Days 18 and 19 of gestation have already been identified in male rats whose mothers were exposed only to stress (Ward & Weisz, 1980, 1984) or only to alcohol (McGivern, Raum, Salido, & Redei, 1988, 1998; Sinha, Halasz, Choi, McGivern, & Choi, 1997). Furthermore, previous studies have shown that the neonatal surge is reduced in male pups by prenatal ethanol exposure (McGivern, Handa, & Redei, 1993; Rudeen & Kappel, 1985). Prior to the present study, possible effects of prenatal restraint stress on the fleeting postparturitional rise in T had not been investigated. Also, no information existed on any portion of the perinatal T pattern in males exposed to both stress and alcohol during fetal ontogeny. Thus, in this study, we compared plasma T in newborn male rats that had been exposed to stress, to alcohol, or to a combination of the two treatments (Ward, Ward, Denning, French, & Hendricks, in press). The objective was to uncover possible shared alterations in T that might be related to the incompletely defeminized behaviors shown by males in all of these groups. Our special interest was to determine whether there were distinct alterations in the timing or size of the postparturitional T surge that might explain the different effects of these treatments on behavioral masculinization.

METHODS

Time-mated female rats were provided with ad libitum access to one of two liquid diets, beginning on Day 10 of pregnancy. In one diet, 36% of the calories consisted of ethanol. In the alternate, isocaloric control diet, alcohol was replaced by maltose dextrin. Intake of the dams was monitored by weighing the drinking tubes in which the diets were presented daily.

Half of the females receiving each of the two diets were stressed from Days 14 to 21 of pregnancy, using a procedure standardized in our laboratory (Ward, 1972a, 1977). Specifically, each animal was inserted into a Plexiglas restrainer over which approximately 2150 lm/m^2 of white light was delivered. Each session lasted 45 min and was repeated three times daily at 4-hr intervals during the animals' night cycle. This treatment is considered to be stressful because it leads to increased plasma levels of corticosterone in both the dams and their fetuses (Ward & Weisz, 1984). The control group against which the effects of the treatments were to be compared was not stressed. Each control female was given daily access to amounts of control diet whose caloric content was equivalent to that consumed spontaneously by an animal in the alcohol or the combination treatment group to which it had been specifically yoked.

Approximately 21.5 days after breeding, the dams were stunned by a sharp blow to the head and decapitated. The litters were quickly delivered by cesarean section. Each pup was cleaned, sexed by inspecting the ano-genital distance, and placed on a heating pad in a humidified chamber. The males of each litter were equally divided into 4 groups, whose blood was to be harvested at 0 (within 10 min), 1, 2, or 4 hr of delivery. Individual pups were decapitated at their assigned sacrifice time. Blood was collected in heparinized capillary tubes that were centrifuged to allow plasma to be extracted. Standard radioimmunoassay (I. L. Ward et al., 1996; Ward et al., 1999) was used to determine plasma T in duplicate 25 μl samples derived from individual pups.

RESULTS AND DISCUSSION

The typical pattern of disruption in adult sexual behaviors induced by prenatal stress, alcohol, or both will be presented alongside the neonatal T data. For tests of feminization, males castrated at 60 days of age were injected with 50 μg estradiol benzoate and 200 μg progesterone before each of four tests with a stud male (Ward et al., 1994). Males that displayed lordosis following at least 20% of the mounts received on two or more tests were considered capable of displaying female sexual behavior. For tests of masculinization, males castrated as adults were implanted with 30-mm Silastic tubes containing T (Ward et al., 1999). These implants produced T levels that were approximately 70% of normal endogenous levels. Males were given 6 tests with receptive females.

Prenatal Stress Alone

As shown in the upper left panel of Fig. 1, males derived from stressed dams retained the capacity for the female receptive pattern. Sixty-one percent of the stressed males showed lordosis compared to less than 10% of the control males. Although the lordosis scores are high, stressed males, as well as males in the other two treatment groups, are clearly distinguishable from females in that none displayed the soliciting component of the estrous pattern, i.e., darting, crouching, ear wiggling (Ward et al., 1994). This incomplete behavioral defeminization in stressed males was accompanied by a partial failure in

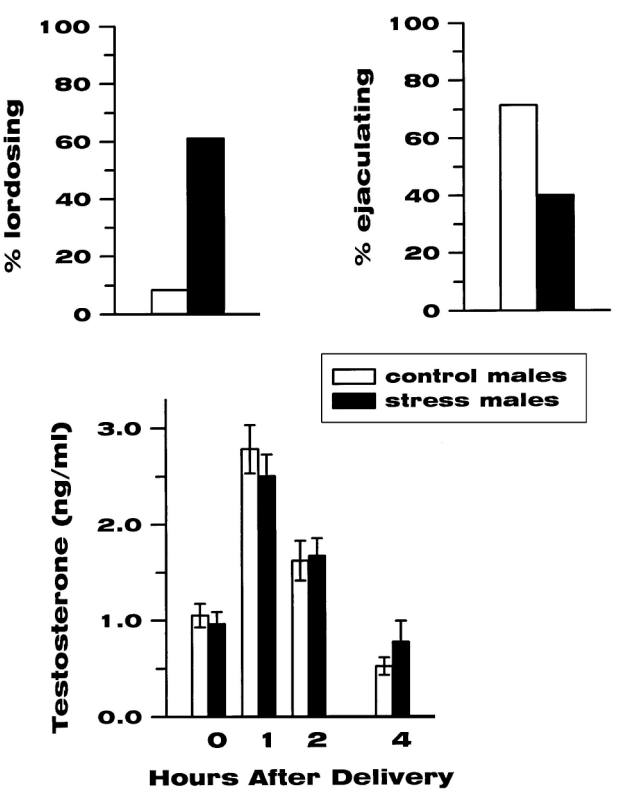

Fig. 1. Typical incidence of the female lordotic (upper left panel) and male copulatory pattern (upper right panel) shown by prenatally stressed male rats (adapted from Ward et al., 1994 and Ward et al., 1999), as compared to their mean ($\pm$*SEM*) postparturitional plasma testosterone levels (lower panel; adapted from Ward et al., in press).

behavioral masculinization (top right panel). Only 40% of stressed males copulated, compared to 75% of control males ($p < .04$). With the exception of two controls, no experimental male copulated without ejaculating. Stressed males that ejaculated did not differ from controls in latency to first mount, number of mounts or intromissions preceding ejaculation, or length of postejaculatory interval (Ward et al., 1999).

Considering the divergence from typical sexual behavior differentiation, it is surprising that prenatally stressed males showed a neonatal T pattern that was not statistically different from that of control males (Fig. 1, bottom panel). Both stressed and control males had a large surge in plasma T after birth. T titers peaked at 1 hr postpartum, declined significantly by 2 hr, and by 4 hr returned to levels not significantly different from those immediately after delivery.

It is interesting that males with a normal neonatal T surge (stress group) nevertheless exhibited an enhanced female behavioral potential. Complete elimination of the T surge within the first hour following birth by orchidectomy (Corbier et al., 1983; Thomas & Gerall, 1969) or by ether administration (Vega Matuszczyk, Silverin, & Larsson, 1990) is sufficient to increase lordosis in males. However, the current results suggest that although suppression of the neonatal surge may be sufficient to augment the female sexual behavior potential, it is not required.

Despite their normal neonatal T profile, prenatally stressed males also show a partial failure in the masculinization of behavior (upper right panel of Fig. 1). As with defeminization, behavioral masculinization can be attenuated by eliminating the postparturitional T surge (Roffi et al., 1987). Yet, the present study clearly shows that alterations in masculinization can occur in males that apparently have had normal neonatal T exposure.

Prenatal Alcohol Alone

As shown in the upper panel of Fig. 2, males exposed to alcohol alone during fetal development showed an enhanced potential to display female receptivity, but displayed normal male copulatory behavior. Thus, in this group sexual behavior differentiation was dissociated. Defeminization was incomplete but masculinization was not affected.

Again, as in the case of stress, the effects of prenatal alcohol on sexual behavior cannot be fully predicted by viewing the postparturitional pattern of plasma T (Fig. 2, bottom panel). T levels in alcohol-exposed males were significantly lower ($p < .04$) than in control males right after delivery (0 hr). Further, although alcohol-exposed

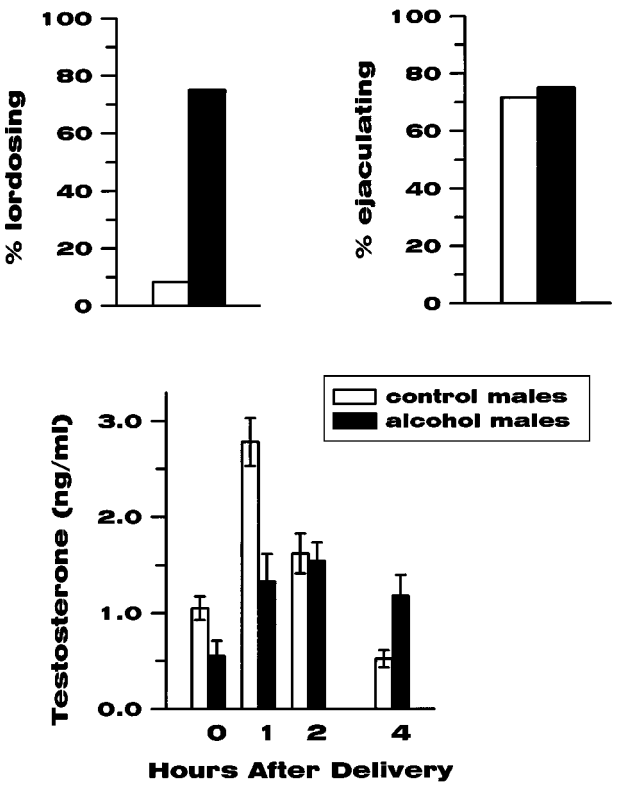

Fig. 2. Typical incidence of the female lordotic (upper left panel) and male copulatory pattern (upper right panel) shown by prenatal alcohol exposed male rats (adapted from Ward et al., 1994 and Ward et al., 1999), as compared to their mean ($\pm SEM$) postparturitional plasma testosterone levels (lower panel; adapted from Ward et al., in press).

males showed a marginally significant increase in plasma T 1 hr after birth ($p < .06$), at that point their T level was only 50% of that seen in control males ($p < .005$). At 2 hr, T remained elevated above the 0-hr level, but it was no longer different from that of the control males who had declined from their 1-hr peak.

The abnormally low postparturient T surge could have contributed to the high lordotic potential of the alcohol-exposed males. But, one important ramification of these data is that, despite the blunted neonatal T surge, alcohol-exposed males showed normal masculine sexual behavior. Thus, normal behavioral masculinization can occur in the face of a neonatal T surge that is reduced by approximately 50%.

Prenatal Alcohol Plus Stress

Adult sexual behavior potentials of males prenatally exposed to both alcohol and stress are incompletely defeminized (Fig. 3, top left panel). Unlike alcohol-alone

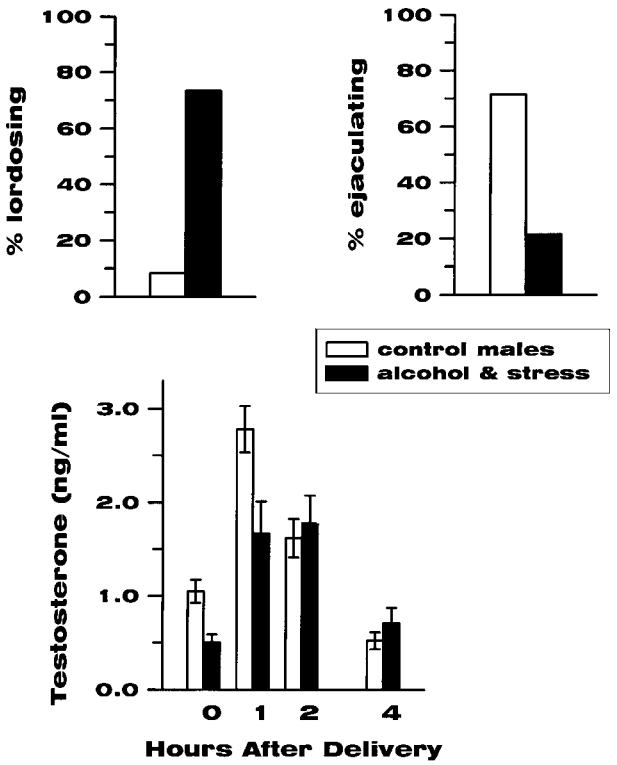

Fig. 3. Typical incidence of the female lordotic (upper left panel) and male copulatory pattern (upper right panel) shown by male rats prenatally exposed both to alcohol and stress (adapted from Ward et al., 1994 and Ward et al., 1999), as compared to their mean ($\pm SEM$) postparturitional plasma testosterone levels (lower panel; adapted from Ward et al., in press).

males, they also have a severe failure in behavioral masculinization (top right panel). Only 21% ejaculated (or even initiated copulation) during six tests with receptive females. For a detailed description of various copulatory parameters associated with the test on which ejaculation occurred in the few responding animals see Ward et al. (1999). It is unlikely that this extreme behavioral deficit can be attributed to inadequate masculinization of the genitalia. Males exposed to both stress and alcohol, like those exposed to stress alone or alcohol alone, show no discernible effects on the dimensions of various reproductive structures such as penile length or weight of the testes or epididymis. They do not retain a blind vaginal opening, as do males prenatally exposed to the androgen receptor blockers, flutamide (Clemens, Gladue, & Coniglio, 1978) or cyproterone acetate (Ward, 1972b). Body weight and ano-genital distance are normal in the combination treatment males (Table I). Thus, sexual behavior potentials were altered without concomitant changes in reproductive structures. All three of these treatments lead to a marked

Table I. Mean Morphological Measures of Adult Male Rats Exposed to Alcohol, Stress, or both, During Gestation (Adapted from Ward et al., 1994)

Prenatal treatment	Body weight (g)	Ano-genital distance (cm)	Penile length (cm)	Testis weight (g)	Epididymis weight (g)
Yoked control	248	3.26	1.02	1.420	.264
Stress	251	3.49	0.97	1.468	.268
Alcohol	255	3.47	0.99	1.364	.257
Alcohol+ stress	250	3.45	0.97	1.407	.254

dissociation between external anatomical features and the sexual behaviors being exhibited.

There are few clues in the neonatal T patterns that would explain why stress-plus-alcohol males show such a severe failure of behavioral masculinization. At delivery (0 hr) stress-plus-alcohol males, like alcohol-alone males, had T levels that were significantly lower than in the control group ($p < .02$). At 1 hr after delivery, plasma T in the stress-plus-alcohol group was significantly below the level of control males ($p < .03$). At no time-point was there a significant difference in the plasma T of the stress-plus-alcohol and the alcohol-alone males. Thus, very similar aberrant neonatal T patterns were associated with quite different effects on the masculinization of adult sexual behavior potentials.

SUMMARY AND CONCLUSIONS

The study replicated the brief surge in plasma T that previously has been reported in male but not female rats during the first hours following birth (Corbier et al., 1978; Lalau et al., 1990).

The objective of this study was to provide insight into the etiology underlying the adult sexual behavior patterns shown by males prenatally exposed to alcohol, stress, or both factors. Given the shared increase in the female lordotic pattern resulting from these prenatal treatments, we expected alterations in the neonatal gonadal hormonal milieu common to all three preparations. We found no such shared alteration. Although circulating T was diminished after birth in both of the groups exposed to alcohol, T was normal in the prenatal stress-alone males. This finding suggests that perusal of the entire perinatal T pattern might yield more fruitful insights into the mechanism underlying behavioral defeminization.

The enhanced lordotic potential in prenatally stressed males may be due entirely to the known disruption in the prenatal T pattern. Prenatally stressed males show higher

than normal levels of plasma T on Day 17 of gestation, but have abnormally low T on each of the following 2 days, a time when control males experience a marked elevation (Ward & Weisz, 1980, 1984). Exposure of developing males to the androgen antagonists flutamide or cyproterone acetate during fetal ontogeny enhances their ability to show increased lordosis (Gladue & Clemens, 1978; Ward, 1972b). However, it is not known whether prenatal treatment with these drugs might not also have affected their neonatal T surge.

Plasma T is attenuated on Days 18 and 19 of gestation in male fetuses of dams fed alcohol during pregnancy (Sinha et al., 1997). Thus, it is likely that the males in both our alcohol-alone and stress-plus-alcohol groups experienced suppressed T during prenatal development. However, we cannot rule out some contribution of the blunted postparturitional T surge to the incomplete behavioral defeminization found in both alcohol-exposed groups. Nevertheless, their lordotic potential was no higher than that of the prenatal stress-alone group. In fact, there were no statistically significant differences in the lordotic potential among males prenatally exposed to alcohol, stress, or both (Ward et al., 1994).

We attempted to identify an alteration in T idiosyncratic to each treatment that might underlie the characteristic male sexual behavior patterns shown by each, particularly the severe decrement characteristic of the combination treatment animals. However, abnormalities in neonatal plasma T were very similar in the two alcohol groups and the pattern of stress-alone males was normal. Thus, deviant postparturitional T patterns alone cannot explain the reduced ability of stress-alone males or the severely diminished capability of stress-plus-alcohol males to copulate with females.

Behavioral masculinization can be partially disrupted by suppression of T confined to the prenatal period. Previous studies have shown that when administration of cyproterone acetate is restricted to Days 13 through 22 (Nadler, 1969), to Days 17 through 19 (Perakis & Stylianopoulou, 1986), or to Days 10 through 19 (Vega Matuszczyk & Larsson, 1995) males show attenuated levels of intromissions and ejaculations. Thus, the incomplete masculinization of dimorphic behaviors that characterized prenatally stressed males can be traced to the prenatal gonadal hormonal milieu, rather than to T circulating during later neonatal stages during which the process of sexual differentiation is completed in the rat (Ward & Ward, 1985).

The pronounced failure in behavioral masculinization seen in stress-plus-alcohol males may involve the entire prepuberal T pattern. The combination treatment group probably experienced the suppressed surge in T shown on Days 18–19 of gestation by males exposed only to stress (Ward & Weisz, 1980, 1984). They do share the reduced postparturitional T surge seen in males exposed to alcohol alone, as revealed by our data. Unfortunately, the prenatal T pattern has not been characterized in stress-plus-alcohol males. Possibly in those fetuses T may already be lower than normal on Day 17 of gestation, a time when stress-alone males experience a premature surge. The existence of such an early abnormality would yield a unique perinatal T pattern that would set the stress-plus-alcohol preparation apart from the other two treatments. Full development of the male sexual potential may require a somewhat different pattern of T exposure than does suppression of female behavior. Our laboratory is currently exploring this possibility.

We have previously postulated (Weisz & Ward, 1980) that sexual differentiation in the male rat requires two sequential phases of T exposure. The first involves the upsurge in T that occurs during prenatal ontogeny. The first priming action sensitizes the brain to the masculinizing action of T circulating during later neonatal stages when sexual differentiation is completed. The sensitizing effect could involve induction of steroid receptors or metabolizing enzymes in various target tissues. We have demonstrated that such a biphasic mechanism may hold for the organization of both sexual behavior (Hoepfner & Ward, 1988) and dimorphic CNS structures (O. B. Ward et al., 1996). If prenatal T exposure in stress-plus-alcohol males were interrupted to a greater extent than in the alcohol-alone group, e.g., starting as early as Day 17 of gestation, it would explain the increased impairment in male copulatory behavior, despite very similar abnormalities in the postparturitional T surge.

In summary, our study underscores the vulnerability of masculinization and defeminization to factors that alter plasma T patterns during early species-specific stages of ontogeny when sexual differentiation of the nervous system and resultant behavioral potentials are ongoing. Although the present paper has focused only on two factors, alcohol and stress, there are many other pharmacological agents commonly used by pregnant women that impact on the pituitary–gonadal axis, e.g., opioids and barbituates (see review by Ward, 1992). Only a few of these substances have received more than cursory attention with regard to their possible impact on the sexual differentiation of behavior in developing fetuses.

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REFERENCES

Baum, M. J., Brand, T., Ooms, M., Vreeburg, J. T. M., & Slob, A. K. (1988). Immediate postnatal rise in whole body androgen content in male rats: Correlation with increased testicular content and reduced body clearance of testosterone. *Biology of Reproduction, 38,* 980–986.

Chen, J. J., & Smith, E. R. (1979). Effects of perinatal alcohol on sexual differentiation and open-field behavior in rats. *Hormones and Behavior, 13,* 219–231.

Clemens, L. G., Gladue, B. A., & Coniglio, L. P. (1978). Prenatal endogenous androgenic influences on masculine sexual behavior and genital morphology in male and female rats. *Hormones and Behavior, 10,* 40–53.

Corbier, P., Kerdelhue, B., Picon, R., & Roffi, J. (1978). Changes in testicular weight and serum gonadotropin and testosterone levels, before, during, and after birth in the perinatal rat. *Endocrinology, 103,* 1986–1991.

Corbier, P., Roffi, J., & Rhoda, J. (1983). Female sexual behavior in male rats: Effect of hour of castration at birth. *Physiology and Behavior, 30,* 613–616.

Dahlgren, I. L., Eriksson, C. J. P., Gustafsson, B., Harthon, C., Hård, E., & Larsson, K. (1989). Effects of chronic and acute ethanol treatment during prenatal and early postnatal age on testosterone levels and sexual behaviors in rats. *Pharmacology, Biochemistry, and Behavior, 33,* 867–873.

Dahlöf, L.-G., Hård, E., & Larsson, K. (1977). Influence of maternal stress on offspring sexual behaviour. *Animal Behaviour, 25,* 958–963.

Dunlap, J. L., Zadina, J. E., & Gougis, G. (1978). Prenatal stress interacts with prepuberal isolation to reduce male copulatory behavior. *Physiology and Behavior, 21,* 873–875.

Gladue, B. A., & Clemens, L. G. (1978). Androgenic influences on feminine sexual behavior in male and female rats: Defeminization blocked by prenatal antiandrogen treatment. *Endocrinology, 103,* 1702–1709.

Hård, E., Dahlgren, I. L., Engel, J., Larsson, K., Liljequist, S., Lindh, A. S., et al. (1984). Development of sexual behavior in prenatally ethanol-exposed rats. *Drug and Alcohol Dependence, 14,* 51–61.

Hoepfner, B. A., & Ward, I. L. (1988). Prenatal and neonatal androgen exposure interact to affect sexual differentiation in female rats. *Behavioral Neuroscience, 102,* 61–65.

Huffman, L., & Hendricks, S. E. (1981). Prenatally injected testosterone propionate and sexual behavior of female rats. *Physiology and Behavior, 26,* 773–778.

Lalau, J.-D., Aubert, M. L., Carmignac, D. F., Gregoire, I., & Dupouy, J.-P. (1990). Reduction in testicular function in rats: II. Reduction by dexamethasone in fetal and neonatal rats. *Neuroendocrinology, 51,* 289–293.

LeVay, S. (1993). *The sexual brain*. Cambridge MA: The MIT Press.

McGivern, R. F., Handa, R. J., & Raum, W. J. (1998). Ethanol exposure during the last week of gestation in the rat: Inhibition of the prenatal testosterone surge in males without long-term alterations in sex behavior. *Neurotoxicology and Teratology, 20,* 483–490.

McGivern, R. F., Handa, R. J., & Redei, E. (1993). Decreased postnatal testosterone surge in male rats exposed to ethanol during the last week of gestation. *Alcoholism: Clinical and Experimental Research, 17,* 1215–1222.

McGivern, R. F., Raum, W. J., Salido, E., & Redei, E. (1988). Lack of prenatal testosterone surge in fetal rats exposed to alcohol: Alterations in testicular morphology and physiology. *Alcoholism: Clinical and Experimental Research, 12,* 243–247.

Nadler, R. D. (1969). Differentiation of the capacity for male sexual behavior in the rat. *Hormones and Behavior, 1,* 53–63.

Perakis, A., & Stylianopoulou, F. (1986). Effects of a prenatal androgen peak on rat brain sexual differentiation. *Journal of Endocrinology, 108,* 281–285.

Rhees, R. W., Kirk, B. A., Sephton, S., & Lephart, E. D. (1997). Effects of prenatal testosterone on sexual behavior, reproductive morphology and LH secretion in the female rat. *Developmental Neuroscience, 19,* 430–437.

Rhees, R. W., Shryne, J. E., & Gorski, R. A. (1990). Onset of the hormone-sensitive perinatal period for sexual differentiation of the sexually dimorphic nucleus of the preoptic area in female rats. *Journal of Neurobiology, 21,* 781–786.

Roffi, J., Chami, F., Corbier, P., & Edwards, D. A. (1987). Testicular hormones during the first few hours after birth augment the tendency of adult male rats to mount receptive females. *Physiology and Behavior, 39,* 625–628.

Rudeen, P. K., & Kappel, C. A. (1985). Blood and brain testosterone and estradiol levels in neonatal male rats exposed to alcohol in utero. *Biology of Reproduction, 32,* 75.

Sinha, P., Halasz, I., Choi, J. F., McGivern, R. F., & Redei, E. (1997). Maternal adrenalectomy eliminates a surge of plasma dehydroepiandrosterone in the mother and attenuates the prenatal testosterone surge in the male fetus. *Endocrinology, 138,* 4792–4797.

Slob, A. K., Ooms, M. P., & Vreeburg, J. T. M. (1980). Prenatal and early postnatal sex differences in plasma and gonadal testosterone and plasma luteinizing hormone in female and male rats. *Journal of Endocrinology, 87,* 81–87.

Thomas, C. N., & Gerall, A. A. (1969). Effect of hour of operation on feminization of neonatally castrated male rats. *Psychonomic Science, 16,* 19–20.

Thomas, D. A., Howard, S. B., & Barfield, R. J. (1983). Influence of androgen on the development of sexual behavior in the rat. II. Time and dosage of androgen administration during the neonatal period and masculine and feminine copulatory behavior in females. *Hormones and Behavior, 17,* 308–315.

Vega Matuszczyk, J., & Larsson, K. (1995). Sexual preference and feminine and masculine sexual behavior of male rats prenatally exposed to antiandrogen or antiestrogen. *Hormones and Behavior, 29,* 191–206.

Vega Matuszczyk, J., Silverin, B., & Larsson, K. (1990). Influence of environmental events immediately after birth on postnatal testosterone secretion and adult sexual behavior in the male rat. *Hormones and Behavior, 24,* 450–458.

Ward, I. L. (1972a). Prenatal stress feminizes and demasculinizes the behavior of males. *Science, 175,* 82–84.

Ward, I. L. (1972b). Female sexual behavior in male rats treated prenatally with an antiandrogen. *Physiology and Behavior, 8,* 53–56.

Ward, I. L. (1977). Exogenous androgen activates female behavior in noncopulating, prenatally stressed male rats. *Journal of Comparative and Physiological Psychology, 91,* 465–471.

Ward, I. L., Bennett, A. L., Ward, O. B., Hendricks, S. E., & French, J. A. (1999). Androgen threshold to activate copulation differs in male rats prenatally exposed to alcohol, stress or both factors. *Hormones and Behavior, 26,* 129–140.

Ward, I. L., & Reed, J. (1985). Prenatal stress and prepuberal social rearing conditions interact to determine sexual behavior in male rats. *Behavioral Neuroscience, 99,* 301–309.

Ward, I. L., & Ward, O. B. (1985). Sexual behavior differentiation: Effects of prenatal manipulations in rats. In N. Adler, D. Pfaff, & R. W. Goy (Eds.), *Handbook of behavioral neurobiology* (Vol. 7, pp. 77–98). New York: Plenum.

Ward, I. L., Ward, O. B., Mehan, D., Winn, R. J., French, J. A., & Hendricks, S. E. (1996). Prenatal alcohol and stress interact to attenuate ejaculatory behavior but not serum testosterone or LH in adult male rats. *Behavioral Neuroscience, 110,* 1469–1477.

Ward, I. L., Ward, O. B., Winn, R. J., & Bielawski, D. (1994). Male and female sexual behavior potential of male rats prenatally exposed to the influence of alcohol, stress, or both factors. *Behavioral Neuroscience, 108,* 1188–1195.

Ward, I. L., & Weisz, J. (1980). Maternal stress alters plasma testosterone in fetal males. *Science, 207,* 328–329.

Ward, I. L., & Weisz, J. (1984). Differential effects of maternal stress on circulating levels of corticosterone, progesterone, and testosterone in male and female rat fetuses and their mothers. *Endocrinology, 114,* 1635–1644.

Ward, O. B. (1992). Fetal drug exposure and sexual differentiation of males. In A. A. Gerall, H. Moltz, & I. L. Ward (Eds.), *Handbook of behavioral neurobiology* (Vol. 11, pp. 181–219). New York: Plenum.

Ward, O. B., Monaghan, E. P., & Ward, I. L. (1986). Naltrexone blocks the effects of prenatal stress on sexual behavior differentiation in male rats. *Pharmacology, Biochemistry, and Behavior, 25,* 573–576.

Ward, O. B., Ward, I. L., Denning, J. H., French, J. A., & Hendricks, S. E. (in press). Postparturitional testosterone surge in male offspring of rats stressed and/or fed ethanol during late pregnancy. *Hormones and Behavior.*

Ward, O. B., Wexler, A. M., Carlucci, J. R., Eckert, M. A., & Ward, I. L. (1996). Critical periods of sensitivity of sexually dimorphic spinal nuclei to prenatal testosterone exposure in female rats. *Hormones and Behavior, 30,* 407–415.

Weisz, J., & Ward, I. L. (1980). Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses and neonatal offspring. *Endocrinology, 106,* 306–316.