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Stereotyped Behavior Elicited by Amphetamine in the Rat: Influences of the Testes

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BEATTY, W. W., A. M. DODGE AND K. L. TRAYLOR. *Stereotyped behavior elicited by amphetamine in the rat: Influences of the testes.* PHARMAC. BIOCHEM. BEHAV. **16**(4) 565–568, 1982.—Castrating male rats in adulthood increased the duration of stereotyped behavior in response to 5 mg/kg injections of d-amphetamine sulfate; exogenous treatment with testosterone propionate (TP) reversed this effect. Ovariectomy in adulthood had no effect on stereotypy, but TP injections reduced stereotyped responding by ovariectomized females. Thus testosterone exerts comparable effects on stereotypy elicited by amphetamine in both sexes. Males castrated at 1, 6 or 10 days of age but not males castrated in adulthood displayed levels of stereotyped behavior comparable to those of ovariectomized females when all animals were given TP in adulthood. Control experiments indicated that age of castration rather than time since castration was the critical factor, implying that secretions of the testes early in life exert effects on systems that regulate the responses of adults to amphetamine.

Sex differences Stereotyped behavior Amphetamine Testosterone Gonadal hormones

THE stereotyped patterns of sniffing, licking and gnawing that result from treatment with direct and indirect dopamine (DA) agonists are more intense and longer lasting in female rats than in males [3,24], but the endocrine factors underlying this sex difference are not yet understood. Several laboratories have examined the possible influences of gonadal steroids, but the results are contradictory. Some researchers report that ovariectomy reduces [20] and estradiol potentiates [7, 13, 20] the display of stereotyped responses after injections of amphetamine or apomorphine; others [10] have found that estradiol has the opposite effect. Still others have observed no alteration after treatment with gonadal steroids [18].

In our laboratory we have found no effect of ovariectomy in adulthood on the degree or persistence of stereotyped behavior after amphetamine or apomorphine treatment [24]. Castrating males in adulthood appeared to potentiate their response to amphetamine although the effect was not statistically significant. One purpose of the present study was to reexamine the effects of manipulating of gonadal hormones in adulthood on stereotyped behavior. The second purpose was to investigate the possible significance of exposure to testicular hormones during the early neonatal period for the sex differences in response to amphetamine that are observed in adults. Organizational effects of gonadal hormones are known to be important in the development of a variety of sex differences in reproductive and nonreproductive behaviors, brain anatomy and physiology as well as the hepatic enzyme systems that are involved in drug metabolism (see [1, 11, 16, 17] for recent reviews).

EXPERIMENT 1

In this experiment we examined the possibility that factors secreted by the testes during the early neonatal period influence the intensity and persistence of stereotyped behavior elicited by amphetamine. Drug effects were studied in males that were castrated at varying times shortly after birth as well as in animals or both sexes whose endocrine state was manipulated in adulthood.

METHOD

Animals

The subjects were albino rats of both sexes born in the laboratory of dams obtained early in pregnancy from the Holtzman Co., Madison, WI. The pregnant females were housed in plastic maternity cages located in a temperature controlled animal room (22±3°C) that was illuminated from 0600–1800. Food and water were freely available.

Procedure

After the mothers delivered their litters, the pups were removed, sexed and randomly reassigned to isosexual groups of 10 each. To minimize disturbance to the animals, all rats in a group received the same neonatal surgical treatment. Since the pups in a treatment condition were drawn from a pool of at least 10 different litters, confounding of treatment and genetic variables was minimal. Males were castrated on Day 1 (Group C1, N=12), Day 6 (Group C6,

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N=11), Day 10 (Group C10, N=10), Day 20 (Group C20, N=12) or left untreated (see [2] for details concerning the surgery). Females were untreated at this time. The animals were weaned at 20 days of age, but continued to live in the maternity cages until 25 days of age when they were transferred to colony cages that housed 6–8 rats. From 26–40 days of age the social play behavior of most of the animals was observed as described elsewhere [2]. From 41 days of age until the completion of the study, the rats were housed in pairs in standard laboratory cages. They had free access to food and water except prior to surgery and during testing for stereotyped behavior. At 85 days of age 27 females and 27 of the previously untreated males were gonadectomized. After a recovery period of 15–18 days, daily SC injections of testosterone propionate (Oreton Propionate, Schering, 1 mg/day) or the peanut oil vehicle (0.10 cc) began for all animals and continued for 7 days. About half of the ovariectomized females and half of the males castrated in adulthood received TP injections (Group OF-TP, N=13 and Group C85-TP, N=14) while the remaining animals in these surgical conditions received injections of the oil vehicle (Group OF-Oil, N=14 and Group C85-Oil, N=13). Intact randomly cycling females (Group IF, N=21) and intact males (Group IM, N=16) received oil injections. All of the males that were castrated prior to weaning received TP injections.

On the following day, 18–20 hours after the seventh hormone injection, all rats received IP injections of 5 mg/kg d-amphetamine sulfate (expressed as the weight of the salt). The rats were placed individually into standard laboratory cages without food or water and rated for stereotyped behavior every 20 min beginning 20 min after injection using the 7 point scale devised by Kelly and Iversen [14]. On this scale: 0=asleep or stationary, 1=active, 2=active with bursts of stereotyped sniffing and rearing, 3=stereotyped sniffing and rearing over a wide area, 4=stereotyped sniffing or head bobbing in one place, 5=stereotyped behavior in one location with bursts of licking or gnawing, 6=continuous gnawing or licking in one place. Since previous studies [3,24] using this technique indicated high interrater agreement, only a single observer was used. This rater (WWB) was unaware of the hormone injections received by the animals or the age at which preweaning castration had been performed. He could distinguish the sex of the subjects and those animals that had been gonadectomized recently.

RESULTS

Preliminary analysis of the data indicated that the time course of stereotyped behavior following amphetamine injection was similar for all 10 groups. Peak intensities of stereotyped behavior were attained 40–80 min after treatment; thereafter stereotyped responding declined at a rate that was similar for all groups. Although the absolute levels of stereotypy were somewhat lower in the present experiment, the pattern of changes with time was similar to that observed in our previous studies (see e.g., [24], Fig. 1).

To simplify exposition the results of the stereotyped behavior ratings are presented as the average cumulative stereotypy score over the 260 min observation period (Fig. 1). Analysis of these stereotypy scores with the Kruskal Wallis test revealed a highly significant effect of treatment condition ($H=54.8$, $p<0.001$). Subsequent pairwise comparisons were performed with Mann Whitney U tests with the level of significance set at 0.05. These analyses indicated that the presence of testosterone at the time of testing decreased the

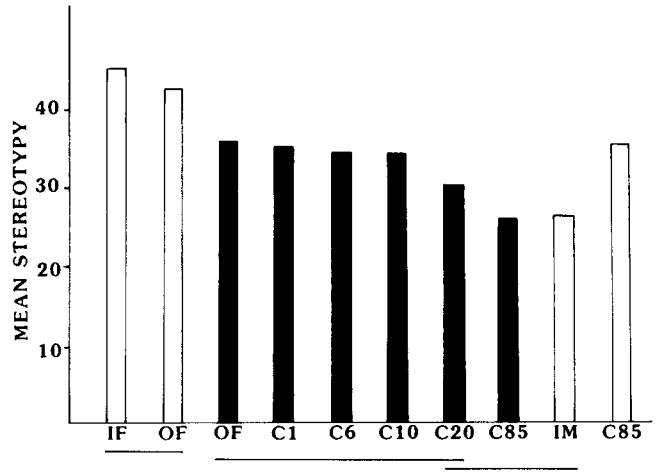

FIG. 1. Mean stereotypy scores cumulated over the 260 min test period in Experiment 1. Open bars indicate oil injections while blackened bars indicate TP injections during the 7 days prior to test. Median scores were as follows: Intact females-oil (IF)=42, Ovx females-oil (OF)=43, Ovx females-TP (OF)=34, C1 males-TP=37.5, C6 males-TP=33, C10 males-TP=35.5, C20 males-TP=30, C85 males-TP=24, Intact males-oil (IM)=26, C85 males-oil=36. Underlined groups did not differ significantly.

display of stereotyped behavior in both males and females. Thus, Group C85-Oil differed from Group IM and Group C85-TP and the latter two groups did not differ. Likewise, ovariectomized females given TP (Group OF-TP) displayed less stereotypy than females that were not given TP (Groups IF and OF oil). These latter groups also did not differ reliably, confirming our earlier observation [24] that the stereotypy scores of randomly cycling intact females are similar to those of ovariectomized females under these test conditions.

The data also suggested an effect of exposure to testicular secretions early in life that could not be reversed by subsequent TP treatment. Thus intact males or males castrated in adulthood and given TP replacement (Group C85-TP), exhibited significantly lower stereotypy scores than males castrated at 1, 6 or 10 days of age and given TP in adulthood. Stereotyped behavior by these neonatally castrated males was quantitatively and statistically similar to that of females given comparable TP treatment in adulthood (Group OF-TP). Males that were not castrated until 20 days of age displayed levels of stereotyped behavior that were intermediate and not significantly different from those seen in neonatal and adult castrates when all groups received TP at testing.

EXPERIMENT 2

The finding in the first experiment that males castrated within the first 10 days of life exhibited higher stereotypy scores than males castrated in adulthood raised the possibility of an effect of neonatal testicular secretions on the physiological mechanisms that regulate responsiveness to amphetamine. However, in that experiment age of castration was confounded with time since castration. Since it is firmly established that lower doses and shorter periods of androgen replacement are required to maintain male reproductive behavior following castration than to restore the behavior once

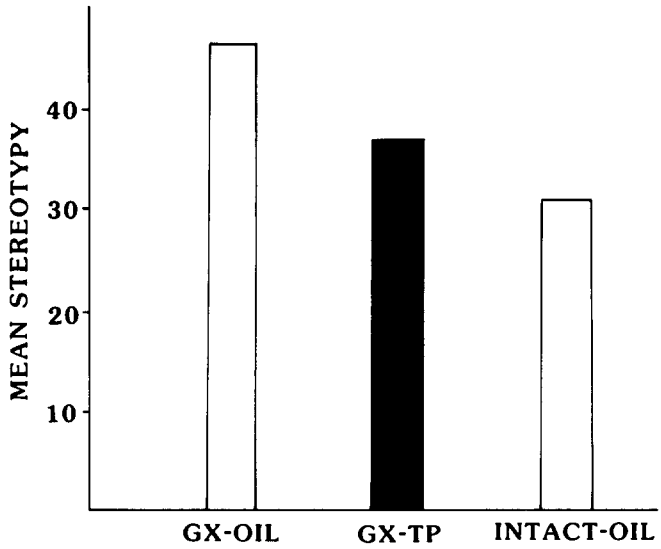

FIG. 2. Mean stereotypy scores cumulated for the 260 min test period in Experiment 2. Median scores were: Gx-oil=44, Gx-TP=36, Intact-oil=30. Group Gx-oil differed from the other two groups which did not differ reliably.

it has waned [21], it might be argued that the apparent effect of exposure to testicular factors early in life inferred from the higher levels of stereotypy observed in groups of male rats castrated early in life was simply the result of an inadequate TP replacement regimen.

To test this possibility we studied stereotyped behavior in male rats that were castrated in adulthood and received TP replacement starting about 3 months after gonadectomy.

METHOD

The animals were 46 male albino rats obtained from the Holtzman Co. at about 70 days of age. At 80 days of age 30 rats were castrated as described elsewhere [24]; the remaining animals were untreated. Ninety-one days later the castrated rats were assigned at random to groups of 15 rats each that received daily SC injections of TP (1.3 mg/day) or an equivalent volume of the oil vehicle (0.13 cc). Intact males (N=16) received oil injections. The absolute TP dose was increased to adjust for the greater body weight of the animals in the present study. On the average males castrated in adulthood received 2.5 mg/kg/day TP in both experiments. Throughout the study the rats were caged in pairs with free access to food and water except prior to surgery and during stereotypy tests. After 7 days of hormone treatment stereotyped behavior following IP injection of 5 mg/kg d-amphetamine sulfate was studied as in the first experiment.

RESULTS

As seen in Fig. 2 castration increased the overall amount of stereotyped behavior and TP injections reversed this effect. A Kruskal Wallis analysis revealed a reliable effect of gonadal condition ($H=18.5$, $p<0.001$). Subsequent analyses with Mann-Whitney U tests confirmed the apparent difference between groups Gx-Oil and Gx-TP ($p<0.02$) and between the intact male group and the oil-treated castrate group ($p<0.001$). The difference between the intact male and Gx-TP groups was not reliable although it approached significance ($p<0.10$).

GENERAL DISCUSSION

As in earlier experiments [3,24] female rats displayed more prolonged stereotyped behavior than males when treated with amphetamine. The present findings suggest that testicular secretions exert influences early in development as well as later in life which may contribute to the sex difference in stereotypy. If present at the time of testing, gonadal androgens reduce the display of stereotypy in both sexes. In the present study males that were castrated in adulthood displayed more stereotyped behavior than intact males while TP injections reduced stereotypy in gonadectomized males and females by about the same degree. In an earlier study [24] castrated males showed a qualitatively similar increase in amphetamine-elicited stereotypy, but the trend was not statistically reliable ($p<0.10$). Since the sample size was twice as large in the present study, this discrepancy seems to be largely a matter of differential statistical power.

It is now recognized that the integrity of the dopaminergic nigrostriatal system is essential for the display of stereotyped behavior after amphetamine treatment [8]. Sex differences have recently been reported in the amphetamine-stimulated release of DA from striatal fragments [4], but it is unlikely that such differences can account for the present findings since DA release is reduced by gonadectomy in females but not in males while stereotypy is altered by castrating males but ovariectomy has no effect, at least under the present conditions.

Hormonal influences on the rate of metabolism of amphetamine provide a more promising explanation for the effects of TP injections in adults on stereotyped behavior. In adult rats brain levels of amphetamine decline more slowly after a single drug treatment in females than in males, apparently because of sex differences in the rate of hepatic parahydroxylation, the major pathway for detoxification in this species [19]. Castrating males in adulthood slows the rate of amphetamine metabolism leading to increased brain levels of the drug [5,19] while TP injections stimulate parahydroxylation in both sexes. Thus the effects of manipulating androgen levels in adulthood on stereotyped behavior can be accounted for rather nicely by the known effects on amphetamine metabolism of these hormones.

The influence of neonatal castration is clearly distinct from that of castration in adulthood since neonatally castrated males and ovariectomized females exhibited more stereotyped behavior than males that were castrated at 85 days of age when all groups received TP at the time of testing. The data from Experiment 2 suggest that differences in amphetamine-elicited stereotypy between the neonatally castrated males and males castrated in adulthood are related to age of castration rather than time since castration, implying that some factor secreted by the testes of the neonate alters the action of amphetamine in the adult. Neither the biochemical identity of this factor nor its mechanism of action are clear at present, but whatever its nature, it evidently must be present for longer than 10 days after birth. This inference is based on the observation that males castrated on day 1, 6 or 10 displayed comparable stereotypy scores that were also comparable to those of females when all subjects received TP injections in adulthood. In this context it is worth noting that early exposure to androgens alters the

hepatic metabolism of many steroid hormones and several drugs as well. In certain cases (e.g., the metabolism of androstenedione by 16α-hydroxylase) extended exposure to androgens early in life is required for the characteristic male enzyme activity to develop [11]. The possibility that products of the neonatal testes alter hepatic systems responsible for parahydroxylation of amphetamine warrants consideration.

Left unresolved by the present findings is the role of ovarian hormones, especially estradiol, in the regulation of stereotyped behavior after amphetamine treatment. Under our testing conditions, we have consistently failed to observe differences in drug-induced stereotypy between ovariectomized and randomly cycling females ([24] and Experiment 1). Other workers report that ovariectomy depresses and estradiol potentiates stereotyped or rotational behavior, responses that are thought to reflect the activity of nigrostriatal DA neurons [7, 8, 12, 13, 15, 20, 21, 22, 23]. Still others have found exactly the opposite, namely that estradiol depresses stereotyped behavior elicited by DA agonists [10].

In an attempt to reconcile these conflicting results, Gordon and his colleagues [9] have suggested that stereotypy may be potentiated or suppressed by estradiol treatment depending on the dose and interval between the hormone and DA agonist treatment. However, as Chiodo *et al.* [7] note, potentiation of the behavioral response to DA agonists has been observed over a wide range of estrogen-test intervals so it is unlikely that variations in dose and test interval can explain all the divergent results. Other, as yet unidentified variables, must also be important. Perhaps this is not surprising since it has recently been shown [6] that there are two distinct populations of nigrostriatal DA neurons which differ in their response to estradiol as well as to environmental stimulation. Differences in the testing environments among laboratories might differentially engage the estradiol-sensitive DA neurons, leading to enhancement or suppression of stereotyped behavior.

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