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# Inhibition of Sexual Behavior by Dopamine Antagonist or Serotonin Agonist Drugs in Castrated Male Rats Given Estradiol or Dihydrotestosterone[1]

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BAUM, M. J. AND M. S. STARR. *Inhibition of sexual behavior by dopamine antagonist or serotonin agonist drugs in castrated male rats given estradiol or dihydrotestosterone.* PHARMAC. BIOCHEM. BEHAV. 13(1) 57–67, 1980.—Four experiments were performed to determine whether the activational effects of two behaviorally active neural metabolites of testosterone, estradiol ($E_2$) and 5α-dihydrotestosterone (DHT), on male rats' sexual behavior possibly result from the action of either steroid at dopaminergic or serotoninergic synapses. In Experiment 1 lower doses of the dopamine receptor antagonist, spiperone, were needed significantly to reduce mounting and intromission rates in castrated males implanted with silastic capsules containing $E_2$ as opposed to DHT. However, in Experiment 2 increasing doses of another dopamine receptor blocker, clozapine, were equally effective in suppressing males' sexual behavior, regardless of whether they were implanted with $E_2$ or DHT, suggesting that these testosterone metabolites may both normally contribute to the activation of masculine sexual behavior by enhancing dopaminergic neurotransmission. In Experiment 3 administering increasing doses of the serotonin reuptake blocker, fluoxetine, caused an equal suppression of sexual behavior in castrated males implanted with $E_2$ of DHT. In Experiment 4 no differential suppressive effects of the serotonin receptor agonist, 5 methoxy-N,N-dimethyltryptamine were obtained in castrated rats implanted with $E_2$ or DHT. It is suggested that these testosterone metabolites may both contribute to the activation of masculine sexual behavior by suppressing activity at serotoninergic synapses.

Male rat  Sexual behavior  Estradiol  Dihydrotestosterone  Dopamine  Serotonin

CASTRATION of the adult male rat causes a decline in sexual performance during the subsequent 4–6 weeks—an effect which is completely reversed by administering testosterone either systemically [6] or directly into the preoptic-anterior hypothalamic regions of the brain [13,22]. In the male rat brain, as in the brains of essentially all mammals studied to date, testosterone, which is secreted by the testes, is metabolized into 5α-dihydrotestosterone (DHT) [25] as well as into estradiol ($E_2$) [31]. Also, separate types of intraneuronal receptors for androgen and estrogen, (reviewed in Vreeburg *et al.* [44]), have been identified in the rat forebrain. Much behavioral evidence (summarized below) suggests that in rats both the 5α-reduced androgenic and the estrogenic metabolites of testosterone act directly in the brain to activate masculine sexual behavior.

The earliest indication that both metabolites might be involved in the regulation of masculine sexual behavior in rats came from studies showing that combined administration of $E_2$ and DHT to castrated males restored their sexual performance as readily as testosterone itself [4, 16, 21]. Additional work has specifically implicated neural estrogenic metabolites of testosterone in this process: (a) Administering $E_2$ by itself to castrated rats stimulated appreciable mounting and intromission behavior as well as occasional ejaculations [4,37]; (b) Either intracerebral [12] or systemic [8] administration of drugs which block the conversion of testosterone to estradiol interfered with the ability of testosterone to activate masculine sexual behavior in castrated rats.

Other experimental evidence suggests that 5α-reduced androgenic metabolites of testosterone also contribute to the activation of masculine sexual behavior in the rat by acting in the central nervous system: (a) Low but measurable levels of masculine sexual behavior were elicited by systemic administration of DHT to castrated rats [3, 33, 47]; (b) DHT stimulates penile growth, including the development of cornified papillae on the surface of the glans penis [5], which may provide sensory inputs during copulation [7]. However, when sensory afferent and autonomic connections between the penis and spinal cord were interrupted by cutting the pudendal nerves, the combination of $E_2$+DHT was still twice as effective as $E_2$ alone in facilitating mounting behavior in castrated rats [23]; (c) Combined administration of $E_2$+DHT also stimulated significantly more mounting behavior than $E_2$ alone in ovariectomized female rats whose

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clitorides were coated with a local anesthetic paste prior to testing [5]. A primary aim of the present experiments was to determine whether the activational effects of either $E_2$ or DHT on males' sexual behavior may be selectively linked to altered transmission at dopaminergic or serotoninergic synapses in the rat brain.

Several lines of evidence suggest that enhanced transmission at dopaminergic synapses facilitate the display of masculine sexual behavior in the male rat. Administration of a low dose (2.5 mg/kg) of L-DOPA plus an inhibitor of peripheral aromatic amino acid decarboxylase activity to castrated male rats maintained on a behaviorally subthreshold dose of testosterone dramatically facilitated the display of all aspects of masculine sexual behavior, and this effect was readily blocked by pretreating males with the dopamine receptor blocker, pimozide [28]. Higher doses of L-DOPA actually disrupted males' sexual behavior [19, 20, 28] for reasons which are unclear. Administering the dopamine receptor agonist, apomorphine, in moderate (10–500 $\mu$g/kg) doses, facilitated masculine sexual performance in gonadally intact [32,41] as well as castrated rats maintained on low doses of testosterone [27]. These facilitatory effects of apomorphine on mating were readily reversed by giving the dopamine receptor blocker, pimozide. Using another approach, Caggiula and coworkers [10] studied the sexual behavior of gonadally intact male rats which had received intraventricular injections of the catecholamine neurotoxin, 6-hydroxydopamine. Immediately after receiving the neurotoxin, males' ejaculation latencies and post-ejaculatory intervals lengthened. More severe behavioral deficits were obtained when the males (a) received acute injections of the tyrosine hydroxylase inhibitor, $\alpha$-methyl-p-tyrosine, or (b) were simply tested with stimulus females which displayed lordosis behavior but no invitational behaviors. These latter behavioral deficits were readily overridden by pinching the males' tails during the testing sessions. In other experiments [2] it was found that peripheral electric shocks readily induced copulation in sexually naive male rats, and that this effect of painful shock was selectively blocked by pretreating the animals with dopamine receptor blockers, but not by noradrenergic receptor blockers. Likewise, Malmnäs [27] reported that manipulation of noradrenergic function via a variety of pharmacologic treatments had little effect on males' sexual performance, further suggesting that in the work of Caggiula *et al.* [10] any deficits in males' sexual behavior caused by 6-hydroxydopamine were due primarily to destruction of dopaminergic neurons.

In all of the studies described above the effect on males' sexual behavior of altering dopaminergic function was studied in rats receiving some degree of testosterone stimulation. Malmnäs [26], however, reported that administering low doses of apomorphine to castrated, adrenalectomized male rats also significantly enhanced the display of both mounting and intromission, and that this effect was readily blocked by pimozide. These findings suggest that one way in which testosterone may normally facilitate masculine sexual behavior is by enhancing transmission at dopaminergic synapses. The question then arises of whether one or both of the neural metabolites of testosterone act in this fashion. This problem was explored in Experiments 1 and 2. Subsequently Experiments 3 and 4 were conducted to determine whether the effects of 5$\alpha$-reduced and estrogenic metabolites of testosterone in male rats possibly result from their differential action at serotoninergic synapses, activation of which is thought to inhibit masculine sexual behavior. (Details are given in the introduction to Experiment 3).

## GENERAL METHOD

### *Subjects*

Male rats (200 g) of the hooded Long-Evans strain were purchased from Charles River Breeding Farms and housed in groups of two in a room in which the lights were off between 12:00 and 24:00. Food and water were available ad lib. After it had displayed ejaculation in at least one test with a sexually receptive female, each male was bilaterally castrated via a single midline incision using ether anesthesia. Three weeks later all rats received SC implants of silastic capsules filled with $E_2$ or DHT (details below), again under ether anesthesia. The same males were used in all four experiments. The female rats used to test males' sexual behavior were bilaterally ovariectomized under ether anesthesia, and were made sexually receptive by SC injections of estradiol benzoate (5 $\mu$g in 0.1 ml sesame oil) 48 hr and progesterone (500 $\mu$g in 0.1 ml sesame oil) 4 hr prior to testing.

### *Steroid Administration*

Following castration the male rats were subdivided into three groups. One group received SC implants of silastic capsules (0.058 in. i.d., 0.077 in. o.d., 30 mm length) containing crystalline $E_2$ diluted 10:1 with cholesterol. Although actual circulating levels of $E_2$ were not measured in these experiments, such implants would be expected to produce plasma $E_2$ levels ranging from 90 to 120 pg/ml [24]. These circulating levels of $E_2$ must be considered pharmacologic, in so far as very little (2–3 pg/ml) $E_2$ is normally detected in plasma of gondally intact, adult male rats [14]. Pilot observations from this laboratory (Myers and Baum, unpublished findings, 1978) showed that these particular silastic implants of $E_2$ activated appreciable mounting and intromission behavior, along with occasional ejaculations in long-term, castrate rats. A second group of castrated males received SC implants of silastic capsules (0.062 in. i.d.; 0.125 in. o.d.; 60 mm length) containing crystalline DHT. Pilot observations had shown that this implant activated low, yet measurable levels of masculine sexual behavior in long-term castrate rats. After Experiments 1 and 2 were completed, these particular DHT implants were removed from animals in this group and were replaced with other silastic capsules (0.058 in. i.d.; 0.077 in. o.d.; 50 mm length) which were also filled with DHT. This was done because of a report (W. G. Bradshaw, personal communication, 1979) that these latter DHT implants were particularly effective in activating masculine sexual behavior in castrated rats. In fact, the sexual performance of castrated rats was very similar, regardless of which size silastic implant of DHT they received. Finally, a third group of castrated male rats received two silastic capsules implanted SC, one containing $E_2$ (0.058 in. i.d.; 0.077 in. o.d.; 30 mm length) and another containing DHT (0.062 in. i.d.; 0.125 in. o.d.; 60 mm length). All capsules were sealed at each end with 5 mm of silastic elastomer, incubated at 23°C for 12 hr in 0.9% saline and at 23°C for one hr in 70% ethanol prior to being inserted under the animals' skin.

### *Behavioral Testing*

*Masculine sexual behavior.* All tests were carried out during the dark phase of the day/night cycle in a room lit only by a dim yellow light. Animals were tested in ten-gallon aquariums (25×47×29 cm) with sawdust bedding. Following adaptation of the male to the test cage for 10 min, a sexually receptive female rat was introduced and the male's mounts with pelvic thrusting, intromissions, and ejaculations were

scored by an observer using either an Esterline Angus or a Rustrak event recorder. Following introduction of the female, males were allowed 30 min to achieve an initial intromission. If this occurred, the male received up to 60 min from the start of the test to ejaculate. Tests were stopped after 30 min if the male failed to intromit within that time. If ejaculation occurred, the male was left in the test arena until the initial intromission of a second copulatory series occurred, whereupon the test was stopped.

In the present experiments there were significant differences in the copulatory performance of castrated males receiving different steroid hormones in the absence of drug treatments. Thus for example, animals given $E_2$+DHT were much more likely to ejaculate than males given either $E_2$ or DHT alone. Yet rats receiving $E_2$ or DHT alone displayed appreciable levels of mounting and intromission behaviors in all 4 experiments. In a previous study [23] the rates of mounting and intromitting were found to be reliable indices of males' sexual performance following manipulation of both their hormonal condition and their penile sensitivity. Therefore in the present studies these two parameters of masculine sexual performance were used as the principle dependent variables. Mounting rate (mounts/min) was determined for each test by dividing the total number of mounts (including mounts with intromission) which a male displayed by the time elapsed between the first mount and the ejaculation (or the end of the test if ejaculation failed to occur in the time allotted). Intromission rate (intromissions/min) was determined for each test by dividing the total number of intromissions which a male displayed by the time elapsed between the first intromission and ejaculation (or the end of the test if no ejaculation occurred).

### *Activity*

The effects of various drug treatments on rats' activity was assessed in tests conducted separately from those of sexual behavior using "Automex" activity monitors (Columbus Instruments, Columbus, Ohio). The monitors were used with the sensitivity set at "8." Individual rats were placed in plastic tubs (35×31×17 cm) with wooden tops and 2 cm of sawdust bedding on the floors. Pilot studies in which the effects of a low dose (1.5 mg/kg) of D-amphetamine on rats' activity were observed suggested that this configuration of the Automex monitors maximized the detection of rats' locomotor activity, as opposed to rearing or grooming behaviors displayed while remaining stationary. The Automex monitors were kept in a sound-isolated chamber equipped with fluorescent lights. Tests of activity were carried out with the chamber lights on during what was normally the dark (afternoon) phase of the rats' day/night cycle. Activity levels were measured for 30 consecutive minutes beginning at the same interval after the injection of a particular drug as for tests of sexual behavior.

### *Statistics*

For each experiment the data on animals' activity in the Automex boxes were subjected to a two-way ANOVA, with repeated observations on one dimension (drug dosage). Subsequent comparisons of the means for different drug dosages obtained within each steroid treatment group were made using Newman-Keuls tests. Mounting and intromission rates were analyzed using nonparametric tests because the occurrence of a large number of zero scores following administration of higher doses of various drugs violated the assumptions underlying the use of parametric statistics. Therefore, for each drug tested, the effect of drug dosage on mounting and intromission rates was evaluated separately for rats receiving the respective steroid treatments using the Friedman two-way analysis of variance by ranks [36]. Subsequent individual comparisons between the 0 dose and other doses of each drug were made using Wilcoxon signed rank tests. In each experiment the mounting and intromission rates of rats implanted with different steroids were compared for tests in which only the drug diluent was administered: Overall between groups comparisons were made using Kruskal-Wallis one-way analysis of variance, and subsequent comparisons between pairs of treatment groups were made using Mann-Whitney U tests.

## EXPERIMENT 1: EFFECTS OF SPIPERONE

As outlined in the introduction, if either $E_2$ or DHT act centrally to facilitate masculine sexual behavior by enhancing neurotransmission at dopaminergic synapses, then the behavioral effects of each steroid might be blocked by acute administration of postsynaptic dopamine receptor antagonists. Spiroperidol (Janssen R54147; spiperone; SPIP) is a highly specific dopamine receptor antagonist which apparently acts on dopaminergic receptors distributed throughout the brain as well as in the anterior pituitary gland. Its ability selectively to attenuate masculine sexual behavior was studied in castrated male rats implanted with different steroids.

### *Method*

The rats used in this experiment included 9 animals implanted with $E_2$ alone, 10 animals implanted with DHT alone, and 8 rats implanted with $E_2$+DHT. Pilot studies showed that rats became severely cataleptic following administration of doses of SPIP greater than 200 μg/kg. Accordingly, the effect of several lower doses of SPIP (0, 25, 50, and 100 μg/kg) on males' sexual behavior and activity levels were studied, beginning 1 hr after IP injection in tartaric acid ($3.33\times10^{-3}$ M, pH=3.5).

An initial series of tests assessed the effects of SPIP on males' sexual behavior. All animals received injections of SPIP or its diluent on alternate weeks (SPIP being given in the sequence of 100, 50, and 25 μg/kg). In the final analysis of these data the sexual performance of each rat under the drug vehicle condition was taken as the mean of that rat's scores for tests in which only diluent was given. This procedure of interspersing tests with diluent with between tests with each dose of SPIP was used instead of a counterbalanced, latin square design in studying the effect of the drug on sexual behavior. It will be seen in the Results that this procedure yielded consistent, dose-dependent changes in sexual performance in all three groups of rats. After tests of sexual behavior were completed, all rats received tests every 3–5 days in the Automex activity monitors. Each animal was tested once after injection of each of the doses of SPIP or its diluent using a latin square design.

### *Results and Discussion*

Administering increasing doses of SPIP caused significant overall reductions in the mounting rates of rats implanted with $E_2$ ($p<0.01$), DHT ($p<0.05$), or $E_2$+DHT ($p<0.01$) (Fig. 1). Significant reductions in mounting rate were obtained with different minimal doses of SPIP in rats maintained on different steroids. The 25 μg/kg dose of SPIP caused signifi-

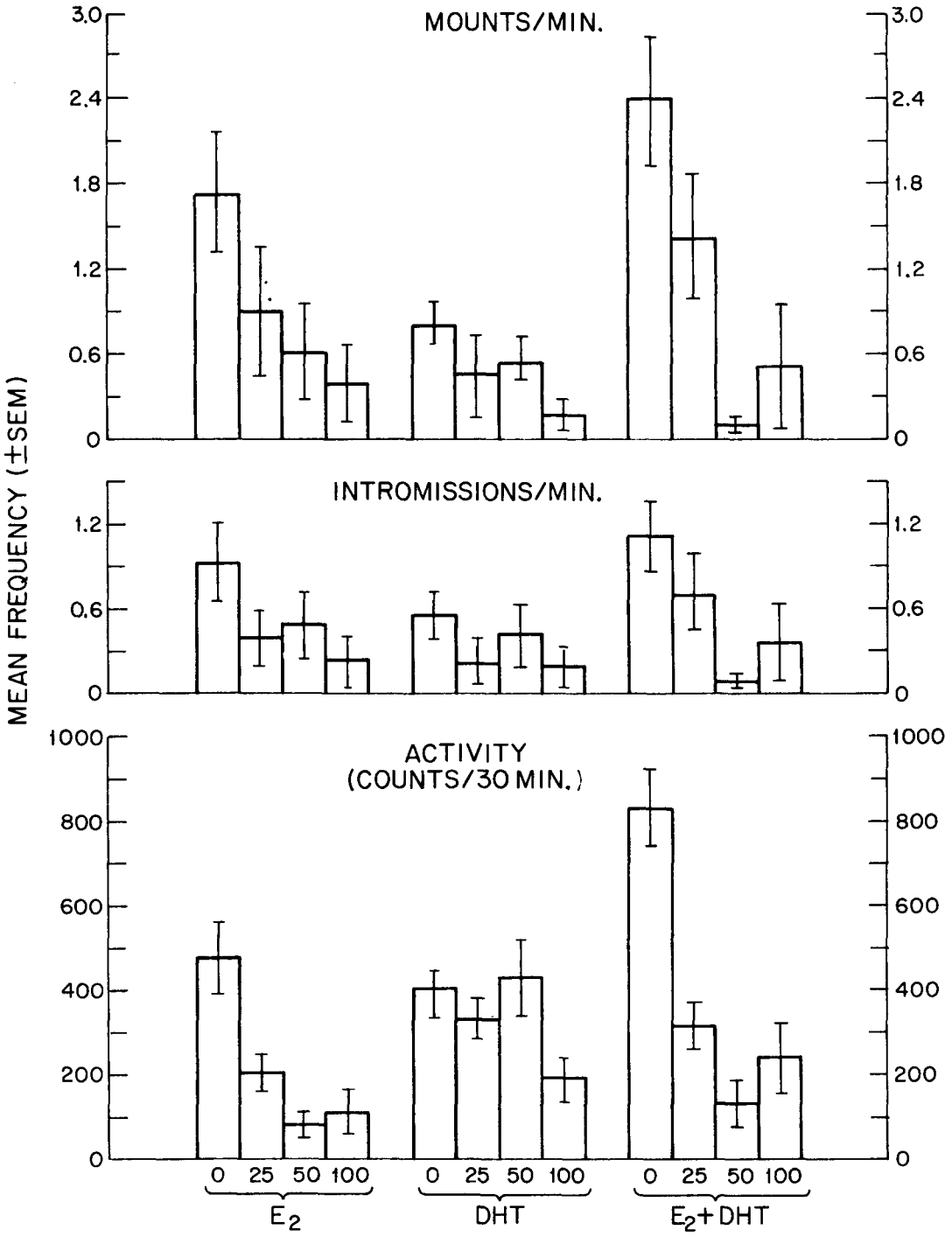


FIG. 1. Effect of administering spiperone (0, 25, 50, or 100 μg/kg) on mounting and intromission rates and on activity levels of castrated male rats implanted SC with silastic capsules containing either $E_2$ (n=9), DHT (n=10), or $E_2$+DHT (n=8).

cant reductions in the mounting rates of the $E_2$ ($p<0.05$) and $E_2$+DHT ($p<0.01$) groups whereas in DHT-treated males 100 μg/kg of SPIP was needed to cause a significant reduction in mounts/min ($p<0.05$). Intromission rates were also reduced significantly after administration of increasing doses of SPIP in rats implanted with $E_2$ ($p<0.05$), DHT ($p<0.05$), and $E_2$+DHT ($p<0.01$). Administration of the lowest dose (25 μg/kg) of SPIP caused significant reductions ($p<0.05$) in intromission rates in all three groups of rats. The higher doses of SPIP caused significant reductions in intromission rates in groups of animals implanted with $E_2$ or $E_2$+DHT. In DHT-implanted males administering 50 μg/kg SPIP failed to reduce intromission rates whereas the 100 μg/kg dose did.

In agreement with previous findings [23], there was a sig-

nificant ($p<0.02$) overall effect of steroid treatment on males' mounting rates in tests when only the drug vehicle was administered. Individual group comparisons showed that mounting rates were significantly lower ($p<0.05$) in males implanted with DHT instead of $E_2$+DHT. There were no other statistically significant effects of steroid treatment on males' sexual behavior.

SPIP-induced reductions in mounting and intromission rates were for the most part paralleled in the three groups by reductions in activity. Thus there was a significant, $F(2,23)=5.55$, $p<0.05$, effect of steroid treatment on activity as well as a significant overall effect of drug dose, $F(3,69)=25.36$, $p<0.01$, and a steroid×drug dose interaction, $F(6,69)=5.93$, $p<0.01$. Individual comparisons within each group of rats showed that for $E_2$ and $E_2$+DHT-implanted animals significant ($p<0.01$) reductions in activity occurred with all doses of SPIP. By contrast, in DHT-treated males only the highest (100 μg/kg) dose of SPIP caused a significant ($p<0.05$) reduction in activity. Thus in DHT-treated animals there was a correlation between the ability of increasing doses of SPIP to cause reductions in mounting rate and reductions in activity. In these rats, however, a significant reduction in intromission rate was obtained with 25 μg/kg SPIP without any reduction in activity. This was the only exception to the general finding in this experiment that drug-induced reductions in sexual performance were correlated with reductions in activity levels.

Inspection of the pattern of results presented in Fig. 1 suggests that SPIP caused significant reductions in mounting, intromission, and activity in rats which received $E_2$, either alone or in combination with DHT, more readily than in animals given DHT alone. This finding could reflect a difference in the action of $E_2$ and DHT on central dopaminergic neurotransmission, or alternatively, it might reflect a differential effect of these steroids on the peripheral metabolism of SPIP. This second possibility is raised by the recent report [11] that administering $E_2$ to ovariectomized female rats caused significant increments in the ability of spiperone to induce catalepsy along with significant increases in blood and brain levels of $^3$H-SPIP following systemic administration of the tritiated drug. The extent to which DHT mimics the action of $E_2$ on SPIP metabolism is unknown; however, it seems possible that in the present experiment the differential effects of SPIP on masculine sexual behavior in rats implanted with $E_2$ as opposed to DHT may have resulted largely from different effects of these steroids on peripheral metabolism of this drug. This does not rule out the possibility that $E_2$ and DHT also differentially affect transmission at central dopaminergic synapses. SPIP is a butyrophenone neuroleptic drug. One way of pursuing the question of whether steroids differentially affect central dopaminergic function was to repeat the present study using another pharmacological class of dopamine receptor blocker.

## EXPERIMENT 2: EFFECTS OF CLOZAPINE

In a second experiment the dibenzazepine neuroleptic, clozapine (CLOZ), which may be especially active as a dopamine receptor blocker at meso-limbic dopaminergic synapses [9], was administered to the rats used in Experiment 1.

### *Method*

One $E_2$-implanted male rat died during the two-week interval between Experiments 1 and 2. In an initial series of tests the effects of administering either 0, 4, 10, or 20 mg/kg of CLOZ on males' sexual performance was studied using a latin square design. CLOZ was injected IP 2 hr prior to behavioral tests. The drug was freshly dissolved in citrate buffer (1.2% citric acid+0.19% sodium citrate dihydrate) just prior to injection. Drug treatments and behavioral tests were given every 3–5 days, with each rat being tested once under each drug dosage. Beginning one week after the last of these tests of sexual behavior, the same series of drug treatments was given to all rats to assess the effects of CLOZ on their activity.

### *Results and Discussion*

Administering increasing doses of CLOZ caused significant overall reductions in mounting rates in castrated rats bearing implants of $E_2$ ($p<0.01$), DHT ($p<0.05$), or $E_2$+DHT ($p<0.01$) (Fig. 2). The minimal dose of CLOZ needed to cause a significant reduction in mounting rate was 4 mg/kg in rats treated with either $E_2$ ($p<0.01$) or DHT ($p<0.05$); a dose of 10 mg/kg CLOZ was required to cause a significant ($p<0.01$) suppression of mounting in animals given $E_2$+DHT. Intromission rates were also reduced significantly after administration of increasing doses of CLOZ in rats implanted with $E_2$ ($p<0.05$), DHT ($p<0.05$), or $E_2$+DHT ($p<0.01$). As with mounting, significant reductions in males' intromission rates were obtained with 4 mg/kg CLOZ in animals given either $E_2$ ($p<0.05$) or DHT ($p<0.05$), whereas a dose of 10 mg/kg was needed to achieve a significant ($p<0.01$) reduction in intromission rates of rats implanted with $E_2$+DHT. Thus in contrast to the effects of SPIP in Experiment 1, the ability of CLOZ to suppress mounting and intromission was nearly identicial in castrated rats given either $E_2$ or DHT. It seems more likely that the differential effects of SPIP on males' sexual performance resulted primarily from steroid-specific differences in the peripheral metabolism of this drug.

As in Experiment 1, there was a significant ($p<0.05$) overall effect of steroid treatment on males' mounting rates during tests in which only drug diluent was given, with mounting rates being significantly higher in males implanted with $E_2$ or $E_2$+DHT as opposed to DHT alone ($p<0.05$). Intromission rates were not significantly affected by steroid treatments in this experiment.

Administering increasing doses of CLOZ caused significant reductions in activity levels in each group of rats. Although there was no significant effect of steroid treatment on animals' activity, $F(2,23)=0.807$, ns, the drug dosage effect was significant, $F(3,69)=23.108$, $p<0.01$. The steroid × drug dosage interaction was not significant, $F(6,69)=0.514$, ns. Subsequent within group comparisons showed that in males implanted with $E_2$ doses of CLOZ as high as 10 or 20 mg/kg were required for significant ($p<0.01$) reductions in activity whereas a lower dose (4 mg/kg) caused a significant reduction in sexual performance. Thus in this group of males drug-induced reductions in sexual performance were not necessarily associated with reduced activity. In males implanted with $E_2$+DHT, as in $E_2$-primed animals, significant ($p<0.05$) reductions in activity were obtained only after higher (10 or 20 mg/kg) doses of CLOZ. In animals implanted with DHT significant ($p<0.01$) reductions in activity were obtained after all three doses of CLOZ. Thus in these rats, as in males implanted with $E_2$+DHT, drug-induced reductions in sexual performance were correlated with reduced activity levels.

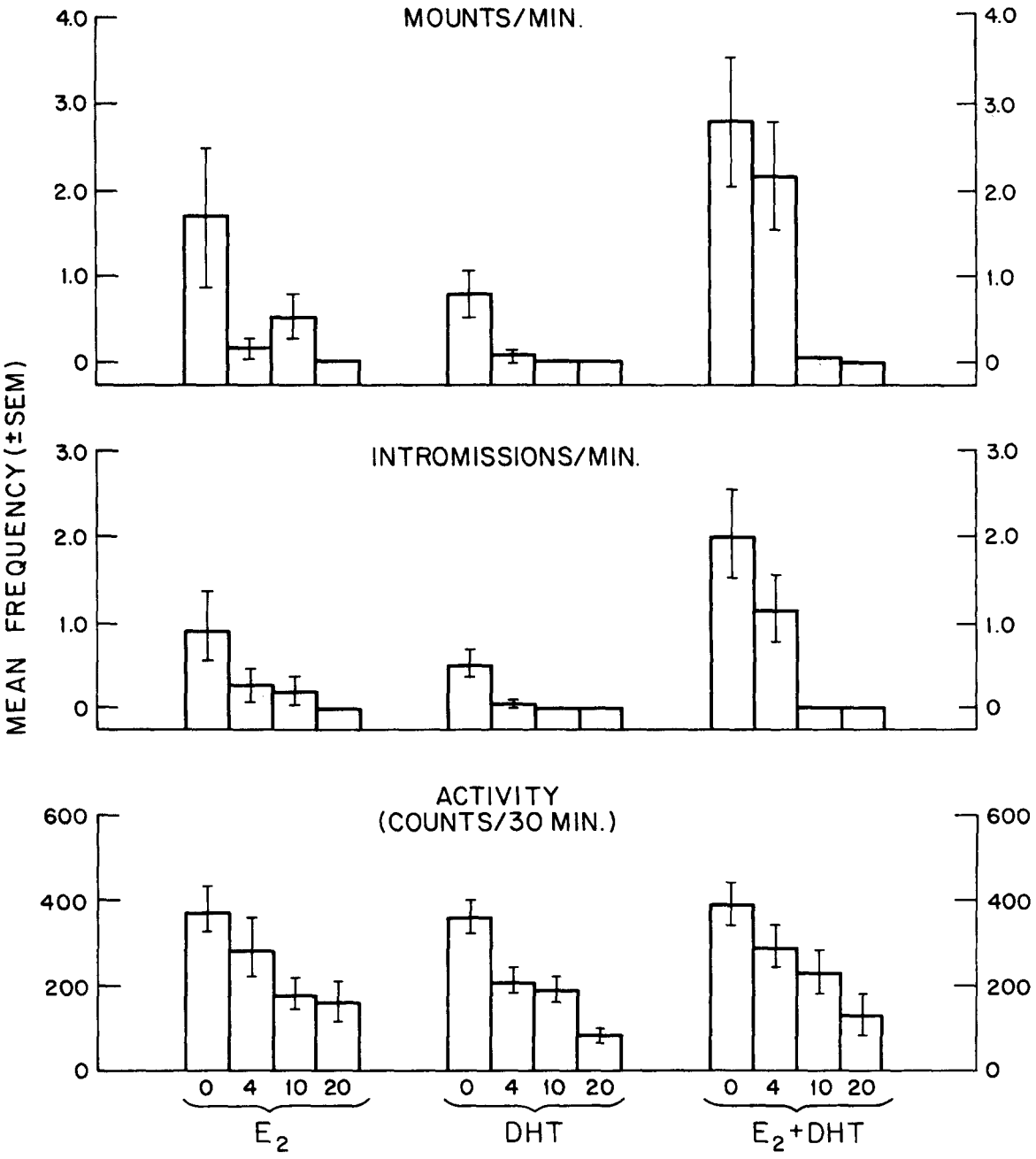


FIG. 2. Effect of administering clozapine (0, 4, 10 or 20 mg/kg) on mounting and intromission rates and on activity levels of castrated male rats implanted SC with silastic capsules containing either $E_2$ (n=8), DHT (n=10), or $E_2$+DHT (n=8).

The results of Experiment 2 suggest that both $E_2$ and DHT contribute to the activation of masculine sexual behavior by enhancing transmission at dopaminergic synapses.

### EXPERIMENT 3: EFFECTS OF FLUOXETINE

Several lines of evidence suggest that transmission at serotoninergic synapses inhibits the display of masculine sexual behavior in the male rat. Administration of p-Parachlorophenylalanine (PCPA), which inhibits tryptophan hydroxylase activity (as to a lesser extent tyrosine hydroxylase activity), to gonadally intact male rats significantly reduced the display of sexual behavior in tests with sexually receptive females [18,35], although some workers [45] failed to obtain such results. Another approach to the same problem was to make male rats sexually sluggish by castrating them and treating them with behaviorally subthreshold doses of testosterone. When given to such rats, PCPA dramatically increased heterosexual mounting behavior [29]. Likewise, administering the monoamine oxidase inhibitor, pargyline, to such rats inhibited their sexual behav-

ior, whereas this effect was counteracted by concurrent administration of PCPA [29].

Disrupting the synthesis of serotonin also facilitated masculine sexual behavior in castrated male rats given no testosterone, suggesting that one way in which testosterone (or its behaviorally active neural metabolites) might normally activate males' sexual behavior is by reducing transmission at inhibitory, serotoninergic synapses. Thus, daily administration of PCPA to long-term castrate rats induced the complete pattern of sexual behavior, including ejaculation [38]. Comparable effects of PCPA on masculine behavior patterns have been obtained in ovariectomized [39] and in ovariectomized, adrenalectomized [15] female rats. Similarly, administering the serotonin neurotoxins, p-chloroamphetamine or 5,7-dihydroxytryptamine, to castrated male rats stimulated ejaculation in the absence of concurrent testosterone treatment [40]. In the experiment in which castrated male rats received PCPA [38] whole brain levels of serotonin were depleted and serotonin synthesis was retarded, although brain dopamine and norepinephrine were also partially depleted. The authors concluded, however, that the facilitatory effect of PCPA on mating resulted primarily from its effect on serotonin synthesis because: (a) administering $\alpha$-methyl-paratyrosine (to inhibit tyrosine hydroxylase) failed to mimic the behavioral effects of PCPA, and (b) administering the serotonin precursor, 5-hydroxytryptophan, but not the dopamine precursor, L-DOPA, effectively blocked the PCPA-induced facilitation of masculine sexual behavior.

If either $E_2$ or DHT activates masculine sexual behavior by depressing functional activity at serotoninergic synapses, the facilitatory action of either steroid on mating might be selectively blocked by acute administration of drugs which either increase the availability of serotonin in the synaptic cleft or directly activate postsynaptic serotonin receptors. The first of these effects can be obtained with the serotonin reuptake blocker, fluoxetine hydrochloride (Lilly 110140; FLUOX) [46]. In Experiment 3 the ability of this drug differentially to suppress masculine sexual behavior was assessed in groups of castrated male rats implanted with $E_2$, DHT, or $E_2$+DHT.

*Method*

Beginning 3 weeks after the completion of Experiment 2, steroid-implanted male rats first received FLUOX injected in 0.9% saline every 3–6 days in doses of 0, 2, 5, 10 mg/kg. The animals received behavioral tests beginning 45 min thereafter. These particular doses of FLUOX were chosen based on their previously demonstrated ability to (a) suppress a reserpine-induced elevation in whole brain levels of 5-hydroxy indole acetic acid [34] and (b) to induce analgesia in rats [30]. The effect of these doses of FLUOX on males' sexual behavior was tested once using a latin square design, whereupon the same sequence of drug treatments was repeated prior to testing animals' activity levels. One week after the last of these tests all animals received one additional dose (20 mg/kg) of FLUOX 45 min prior to a final test of sexual behavior followed one week later by another injection of FLUOX (20 mg/kg) and a final test of activity.

*Results and Discussion*

Increasing doses of FLUOX caused significant ($p<0.05$) reductions in mounting and intromission rates in rats implanted with $E_2$, DHT, or $E_2$+DHT (Fig. 3). In the case of males implanted with $E_2$ or DHT only, the highest dose (20 mg/kg) of FLUOX was required for a significant ($p<0.05$) reduction in mounting and intromission rates. The next lower dose (10 mg/kg) of FLUOX was the minimal dose needed to reduce significantly mounting and intromission rates in animals implanted with $E_2$+DHT. Although during tests with the drug vehicle, DHT-treated males tended to mount and intromit at lower rates than animals in the other two groups, these differences did not reach statistical significance.

There was no significant overall effect of steroid treatment on activity levels, $F(2,22)=0.265$, ns. The drug dosage effect was significant, $F(4,88)=10.335$, $p<0.01$; however, the steroid $\times$ drug dosage interaction was not, $F(8,88)=0.586$, ns). Subsequent within group comparisons showed that in males implanted with $E_2$ even the highest dose of FLUOX failed to cause a significant reduction in activity. In males implanted with DHT significant reductions in activity occurred after administration of 10 or 20 mg/kg FLUOX, whereas in males implanted with $E_2$+DHT a significant reduction in activity was obtained only with the highest (20 mg/kg) dosage. Thus in castrated males implanted with $E_2$ or $E_2$+DHT, FLUOX caused significant reductions in sexual performance which were not necessarily associated with significant reductions in general activity.

## EXPERIMENT 4: EFFECTS OF 5-METHOXY-N,N-DIMETHYLTRYPTAMINE

In Experiment 3 administering the serotonin reuptake blocker, FLUOX, inhibited sexual behavior at equivalent dose levels in castrated rats, regardless of whether they were implanted with $E_2$ or DHT. These findings support the conclusion that both metabolites may contribute to the activation of males' sexual behavior by suppressing the functional activity of serotoninergic synapses; furthermore, they provide no indication that $E_2$ and DHT are differentially active in this regard. Before accepting the latter conclusion, it seemed desirable to test the ability of yet another serotonin agonist drug to suppress sexual behavior in castrated rats implanted with different steroids. 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) which is a specific serotonin receptor agonist [17] was chosen for this experiment.

*Method*

Several male rats from each treatment group died during the month which intervened between Experiments 3 and 4. The surviving males included 6 animals implanted with $E_2$, 8 rats implanted with DHT, and 4 animals implanted with $E_2$+DHT. All animals received IP injections of 5-MeO-DMT (purchased from Sigma Chemical Co., St. Louis, MO) in doses of 0, 250, 500, or 1000 $\mu$g/kg dissolved in 0.9% saline—2% ethanol every 5–8 days, 15 min prior to behavioral testing. This range of doses was chosen based on the report [42] that the highest of them caused myoclonic movements in rats which had been pretreated with 5,7-dihydroxytryptamine whereas no such response occurred in neurologically intact animals over this dose range of 5-MeO-DMT. Thus it was anticipated that within this dose range 5-MeO-DMT would activate serotonin receptors without stimulating myoclonic movements which would have in themselves been incompatible with mating.

The effects of 5-MeO-DMT on males' sexual performance and activity levels were tested separately using the same

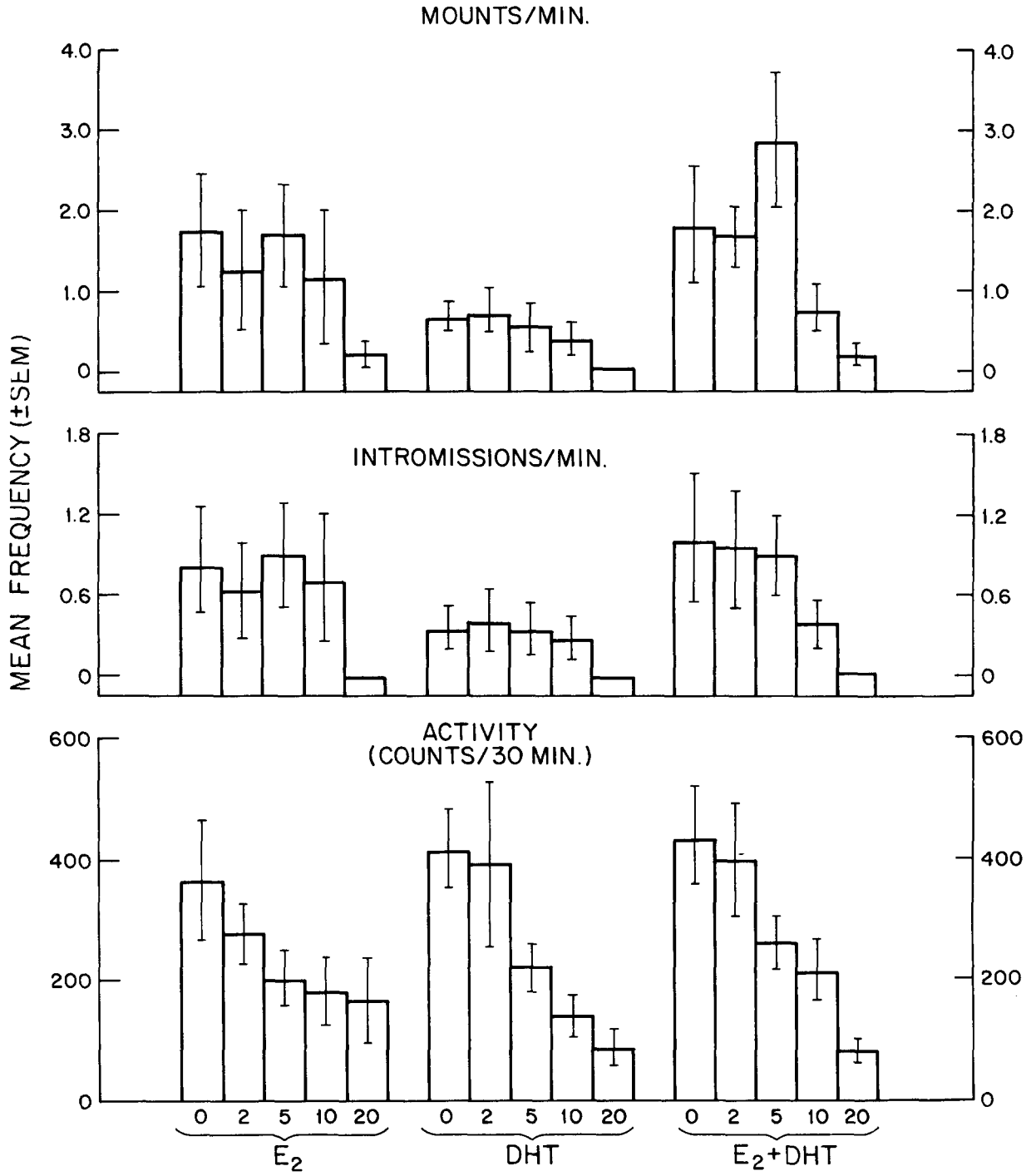


FIG. 3. Effect of administering fluoxetine (0, 2, 5, 10 or 20 mg/kg) on mounting and intromission rates and on activity levels of castrated male rats implanted SC with silastic capsules containing either $E_2$ (=8), DHT (n=10), or $E_2$+DHT (n=8).

Latin squares design twice in succession. Animals' sexual behavior and activity were each tested once after each dose of 5-MeO-DMT. After the last behavioral test all rats were weighed, killed by decapitation, and the seminal vesicles removed and weighed.

*Results and Discussion*

Administering increasing doses of 5-MeO-DMT had no significant effect on mounting or intromission rates in any group of rats, although in males implanted with $E_2$+DHT these parameters tended to decline in response to the highest

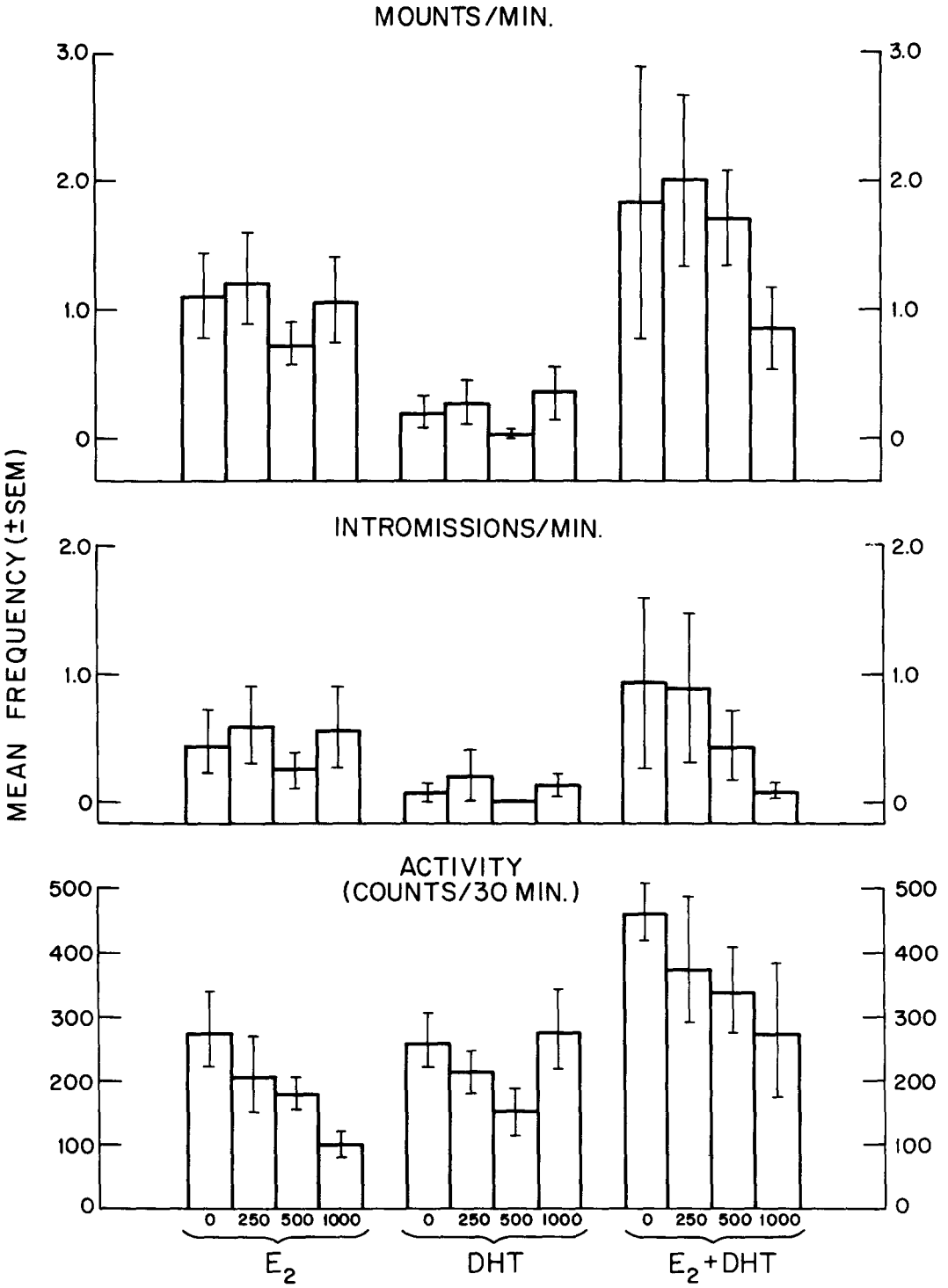


FIG. 4. Effect of administering 5-methoxy-N,N-dimethyltryptamine (0, 250, 500, or 1000 μg/kg) on mounting and intromission rates and on activity levels of castrated male rats implanted SC with silastic capsules containing either $E_2$ (n=6), DHT (n=8), or $E_2$+DHT (n=4).

drug dosage (Fig. 4). To the extent that there was no differential effect of 5-MeO-DMT on the sexual performance of castrated rats implanted with $E_2$ instead of DHT, these findings corroborate those of Experiment 3.

During tests in which only drug diluent was given there was a significant ($p<0.05$) overall effect of steroid treatment on mounting rate, with males implanted with DHT alone displaying significantly lower rates than animals in the other two groups. In this experiment there was no significant overall effect of steroid treatment on intromission rate.

There was a significant overall affect of steroid treatment on males' activity levels, $F(2,15)=9.76$, $p<0.01$; however, neither the drug dosage, $F(3,45)=1.05$, ns, nor the steroid × drug dosage interaction effect, $F(6,45)=1.0$., ns, reached significance. Individual comparisons showed that only in males implanted with $E_2$ was there a significant drug-induced reduction ($p<0.05$) in activity, which occurred in response to the highest (1000 μg/kg) dose of 5-MeO-DMT. In contrast, this dose of drug failed to affect significantly the sexual behavior of rats in this (or any other) group.

At autopsy the body weights of animals implanted with $E_2$, DHT, or $E_2$+DHT were 407±22, 532±21, and 468±40 g (mean±SEM), respectively, $F(2,15)=6.97$, $p<0.01$. Subsequent comparisons showed that only the body weights of the groups implanted with $E_2$ and DHT differed significantly from each other ($p<0.01$). Weights of the seminal vesicles (expressed in mg/100 g) for the same groups were: 21.7±1.9, 121.0±9.4, and 116.3±28.1, $F(2,15)=19.82$, $p<0.01$. Subsequent comparisons showed that seminal vesicles of $E_2$-implanted males were significantly lighter ($p<0.01$) than those of animals in the other two groups.

## GENERAL DISCUSSION

The demonstration in Experiments 1 and 2 that administering neuroleptic drugs reduced sexual performance in male rats complements studies (reviewed in the general introduction) showing that administering drugs which enhance transmission at dopaminergic synapses facilitated masculine sexual behavior. It has been suggested [26] that one way in which testosterone normally contributes to the activation of males' sexual behavior is by enhancing dopaminergic neurotransmission. The present results suggest that the neural metabolites of testosterone, $E_2$ and DHT, both may contribute to this effect at dopaminergic synapses. In an experiment carried out recently in this laboratory [1] castration of adult male rats caused a significant decline in the levels of dopamine as well as in its neural metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, in the n. accumbens and septum, but not in the caudate-putamen. Additional research (L. M. Alderson and M. J. Baum, in preparation) has demonstrated that either $E_2$ or DHT, in addition to testosterone itself, effectively reversed the castration-induced reduction in mesolimbic dopamine content. These neurochemical results, together with the present behavioral findings, suggest that both estrogenic and 5α-reduced androgenic metabolites of testosterone may augment dopaminergic neurotransmission, and thereby contribute to the activation of males' sexual behavior.

The results of Experiment 3 provide additional support to a growing body of literature (reviewed in the introduction to Experiment 3) showing that activation of serotoninergic synapses suppresses sexual behavior in the male rat. As suggested by Södersten and coworkers [38,40], one way in which testosterone may normally activate males' sexual behavior is by reducing transmission at serotoninergic synapses. Indeed, castration of male rats has been found to increase the levels of serotonin [43] as well as its principle neural metabolite, 5-hydroxyindole acetic acid (M. S. Erskine, J. I. Marcus, W. G. Bradshaw, and M. J. Baum, unpublished findings) in certain hypothalamic nuclei. It is not known whether estrogenic and 5α-reduced metabolites of testosterone are both effective in reversing these effects of castration. The results of Experiments 3 and 4 suggest, however, that to the extent that these two metabolites of testosterone normally contribute to the activation of masculine sexual behavior by inhibiting transmission at serotoninergic synapses, they are both active.

A comparison within each experiment of the minimal dose of drug needed to cause a significant reduction in mounting (or intromission) rates in males implanted with $E_2$+DHT as opposed to $E_2$ or DHT alone reveals a different pattern in Experiments 1 and 2, in which dopamine receptor antagonists were given, compared with Experiments 3 and 4, in which serotonin agonists were given. Thus in Experiment 1 the dose of SPIP needed to inhibit mounting in males implanted with $E_2$+DHT was the same as in animals implanted with $E_2$ alone, and both of these groups were more sensitive to the inhibitory effects of the drug than were rats implanted only with DHT. In Experiment 2 more CLOZ was needed to reduce mounting rates in males implanted with $E_2$+DHT than in males implanted with either $E_2$ or DHT alone. The reverse relationship was obtained in Experiments 3 and 4: Less FLUOX was needed to reduce mounting rates in males implanted with $E_2$+DHT than in animals implanted with only $E_2$ or DHT (Experiment 3), and a similar trend was obtained in Experiment 4 following administration of 5-MeO-DMT. The possible functional significance of these differing patterns of drug response remains to be determined.

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