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The antiepileptic primidone impairs male rat sexual behavior

Ashley T. Westerman [a,b], Peter G. Roma [a,c], Evan T. Creed [a], Zachary E. Hurwitz [a], Juan M. Dominguez [a,b,d,*]

[a] Department of Psychology, American University, Washington, DC, 20016, United States
[b] Department of Psychology, The University of Texas at Austin, Austin, TX, 78712, United States
[c] Institutes for Behavior Resources, Baltimore, MD, 21218, United States
[d] The Institute for Neuroscience, The University of Texas at Austin, Austin, TX, 78712, United States

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ABSTRACT

Many antiepileptic drugs (AEDs) produce sexual impairments. Of commonly prescribed AEDs, primidone produces the greatest impairments. Here we examined the effects of primidone on male rat sexual behavior. Sexually-experienced male rats received administration of either vehicle or primidone. After baseline measures were obtained, the effects of daily primidone treatment on home cage sexual performance were assessed three times over the course of 14 days. Motor activity and sucrose preference were also assessed during this time period. Results indicate that primidone impaired copulation but not sexual motivation. Specifically, animals receiving primidone displayed fewer ejaculations, required more time to achieve an intromission, and displayed fewer intromissions per attempted mount as evidenced by a lower intromission ratio. However, animals treated with primidone also chose a goal box containing a sexually-receptive female in an x-maze as often as animals receiving vehicle. The lower intromission ratio suggests an inability to achieve intromissions perhaps as a result of impaired erectile function. Primidone did not affect motor activity or sucrose consumption, an additional measure of natural reward. Together, these data indicate that primidone impairs male sexual activity and suggest that these impairments result primarily from changes in erectile function and not changes to mechanisms mediating motivation.

1. Introduction

Several drugs are currently used to treat epilepsy. These include valproic acid, carbamazepine, phenytoin, benzodiazepines, phenobarbital, and primidone (Yamatogi, 2004). First introduced in the early 1950s (Game, 1953; Ceranke-Hofermayer, 1953; Burton-Bradley, 1953), primidone works primarily via its active metabolite phenobarbital (Löscher and Hönack, 1989). Phenobarbital enhances GABAergic inhibition of the nervous system (White, 1999). A major side effect of primidone is impaired sexual function. In a comparison of the efficacy of the AEDs carbamazepine, phenobarbital, phenytoin, and primidone, Mattson et al. (1985) found that patients taking primidone were more likely to report impotence or decreased libido than were patients taking the other three drugs.

In humans, it is unknown whether sexual dysfunction associated with AEDs is a consequence of impaired desire or fear and social inhibition due to changes in erectile function. An animal model of primidone-induced sexual dysfunction allows us to address the biological bases of this issue by circumventing the complex cultural and interpersonal factors that confound human self-report studies of sexual dysfunction. Additionally, a rat model enables us to parse out the appetitive and consummatory aspects of sexual behavior and examine these different mechanisms individually. For a detailed discussion of the history and use of this locution see previous publications (Ball and Balthazart, 2008; Pfaus, 1999; Pfaus et al., 1990; Sachs, 2007a,b).

Here we describe effects of primidone on sexual function in neurologically healthy male rats. We hypothesized that primidone affects copulation and sexual motivation. Tests also determined the extent to which primidone affected other motivated behaviors, specifically sucrose preference.

2. Method

2.1. Animals

All animals were individually housed in large polycarbonate bins (26×48×21 cm) in a climate-controlled colony room (23 °C). The room was maintained on a reverse 14/10-h light/dark cycle, with lights off at 11 A.M. Food and water were freely available.

Eighteen adult male Long–Evans/Blue Spruce rats (Harlan Sprague Dawley, Indianapolis, IN) were allowed to mate with a sexually-receptive female during three 1-h sessions. During a fourth session, they were allowed to mate for 30 min. Males displaying at least one ejaculation in the 30-min test were randomly assigned to either the vehicle ($n=5$) or primidone group ($n=6$), those not reaching threshold were excluded.

* Corresponding author. Department of Psychology, The University of Texas at Austin, 1 University Station A8000, Austin, TX 78712, United States. Tel.: +1 512 471 1157; fax: +1 512 471 6175.
E-mail address: dominguez@psy.utexas.edu (J.M. Dominguez).

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Thirty female rats were ovariectomized under ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg) anesthesia. They were brought into behavioral estrus with 4 μg of estradiol benzoate at 48 h before and 400 μg of progesterone 4 h before they were used as stimulus females. Behavioral receptivity was confirmed by placing the female with a stud male before they were used in an experiment.

All testing took place between 1 P.M. and 4 P.M. under red light illumination. All procedures were in compliance with the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee at American University, Washington, DC.

2.2. Drugs and solutions

Primidone (Sigma Aldrich, St. Louis, MO) was suspended at a concentration of 25 mg/ml in a 1% Tween-80 solution and administered intraperitoneally (IP) at a dose of 50 mg/kg. This dose was chosen because it has demonstrated efficacy in the treatment of amygdala-kindled rats experiencing seizures (Löscher and Hönack, 1989). For stimulus females, estradiol was suspended at a concentration of 0.02 mg/ml in sesame oil and administered subcutaneously at a dose of 0.2 cc. Progesterone was suspended at a concentration of 2 mg/ml in sesame oil and administered subcutaneously also at a dose of 0.2 cm^3. Consumer-grade sucrose was mixed with tap water at a concentration of 1% (w/v).

2.3. Experimental schedule

Several behavioral variables were measured at baseline and in response to acute and chronic treatment with primidone. Baseline measures were obtained one week prior to the initiation of drug administration. After baseline measures were obtained, the effects of daily primidone treatment on home cage sexual performance were assessed three times over the course of 14 days. Effects of primidone on motor activity were also assessed shortly before animals mated. To determine the generality of primidone's effects on other natural rewards, home cage sucrose preference tests were conducted during the chronic treatment regimen. Finally, following a post-chronic washout, the effects of acute primidone on sexual motivation were further assessed using an x-maze behavioral testing paradigm (cf. Hull et al., 1991).

2.3.1. Sexual activity

Following baseline, primidone's effects on sexual behavior were tested. On test days 1, 7, and 14 of chronic drug administration, males were transported in their home cages into a separate testing room and injected with 50-mg/kg primidone or vehicle. Fifteen minutes after receiving injections, locomotor activity was assessed as described below. Receptive females were then placed in the males' home cages 30 min post-injection and animals were allowed to copulate for 30 min after their first intromission, or if no intromissions occurred, for 30 min after introduction of the female. On non-test days (days 2–6 and 8–13), each animal received its respective injection as described above but was immediately returned to the animal colony.

The following behavioral measures were recorded for all sexual activity tests: mount latency (ML, latency to first mount, or first intromission if not preceded by a mount); intromission latency (IL, latency to the first intromission); ejaculation latency (EL, latency to the first ejaculation); mount frequency (MF_1, number of mounts preceding the first ejaculation); intromission frequency (IF_1, number of intromissions preceding the first ejaculation); intromission ratio [IR = IF / (IF + MF)]; and ejaculation frequency (EF) for the entire test. Measures were recorded on a notepad and then transferred into a computer for analyses. An experimenter blind to experimental conditions observed the behaviors.

2.3.2. Motor activity

In order to account for potential drug-induced motor impairments, we assessed primidone's effects on gross locomotor activity preceding the sexual performance tests. Before each test, a piece of masking tape was placed vertically down the outside center of the males' home cages, and a rater counted the number of times each animal's forepaws crossed the line. These observations were performed for a period of 5 min, beginning 15 min after injection.

2.3.3. Sexual motivation

Two weeks after the sexual performance tests ended, primidone's acute effects on sexual motivation were tested in an x-maze. The x-maze was a cross-shaped plastic apparatus consisting of two 24 × 30 × 23-cm chambers and two 38 × 30 × 30-cm chambers at the end of each of four 49 × 19 × 23-cm arms; entry into each chamber was blocked by a vertically sliding door. One chamber contained an unfamiliar male, while the goal chamber, at the end of the arm opposite the unfamiliar male's chamber, contained a sexually-receptive female.

The subject male was placed in the center of the maze at the beginning of each trial. Upon the subject male choosing a chamber as indicated by forepaws crossing a line drawn 5 cm from the chamber door, an experimenter lifted the door and the male was allowed to enter the chamber. If the male chose one of the two empty chambers or the chamber containing another male, he was confined to the chamber for 1 min. If the male chose the female's chamber, he was confined to the chamber for 5 min or until he achieved an intromission. The sexually-experienced males were trained, drug-free, in the x-maze until they chose the goal chamber on at least 70% of trials during a single session. On test day, they received the same injection of 50-mg/kg primidone ($n = 6$) or vehicle ($n = 5$) 30 min before testing. They were then placed in the center of the maze and choices were recorded. Subject males faced a different clockwise direction each time they were placed in the center of the maze. The test session ended if the male ejaculated or after three consecutive 5-min trials in the goal chamber without an intromission.

2.3.4. Sucrose preference

In order to test the generality of primidone's effects on other natural rewards, a series of sucrose preference tests were also conducted during the chronic treatment regimen (for a review of sucrose preference and food reward, see e.g. Drewnowski, 2005; Berridge, 1996; Sclafani, 2004). For these tests, the regular home cage water bottles were removed, and each male received simultaneous access to a 1% sucrose solution or vehicle tap water in their home cage for 2 h (12 to 2 P.M.). Relative locations of the sucrose and water bottles on their home cages were counterbalanced within each drug treatment group and alternated daily. Both fluids were presented at room temperature in inverted 50-ml tubes and measured to the nearest 0.5 ml. Sucrose preference tests were conducted daily throughout treatment (except for days when sexual behavior was assessed).

2.4. Data analysis

Main and interaction effects of primidone treatment across days and test sessions were analyzed by mixed analysis of variance (ANOVA) models as detailed below. To accommodate homogeneity of variance violations, Mann–Whitney *U* and Wilcoxon signed-ranks tests were employed for post-hoc comparisons. Statistical significance was set at $\alpha = 0.05$ for all analyses.

3. Results

3.1. Sexual activity

Measures of sexual performance were analyzed by 5 × 2 mixed ANOVAs with a repeated-measures factor of test session (baseline,

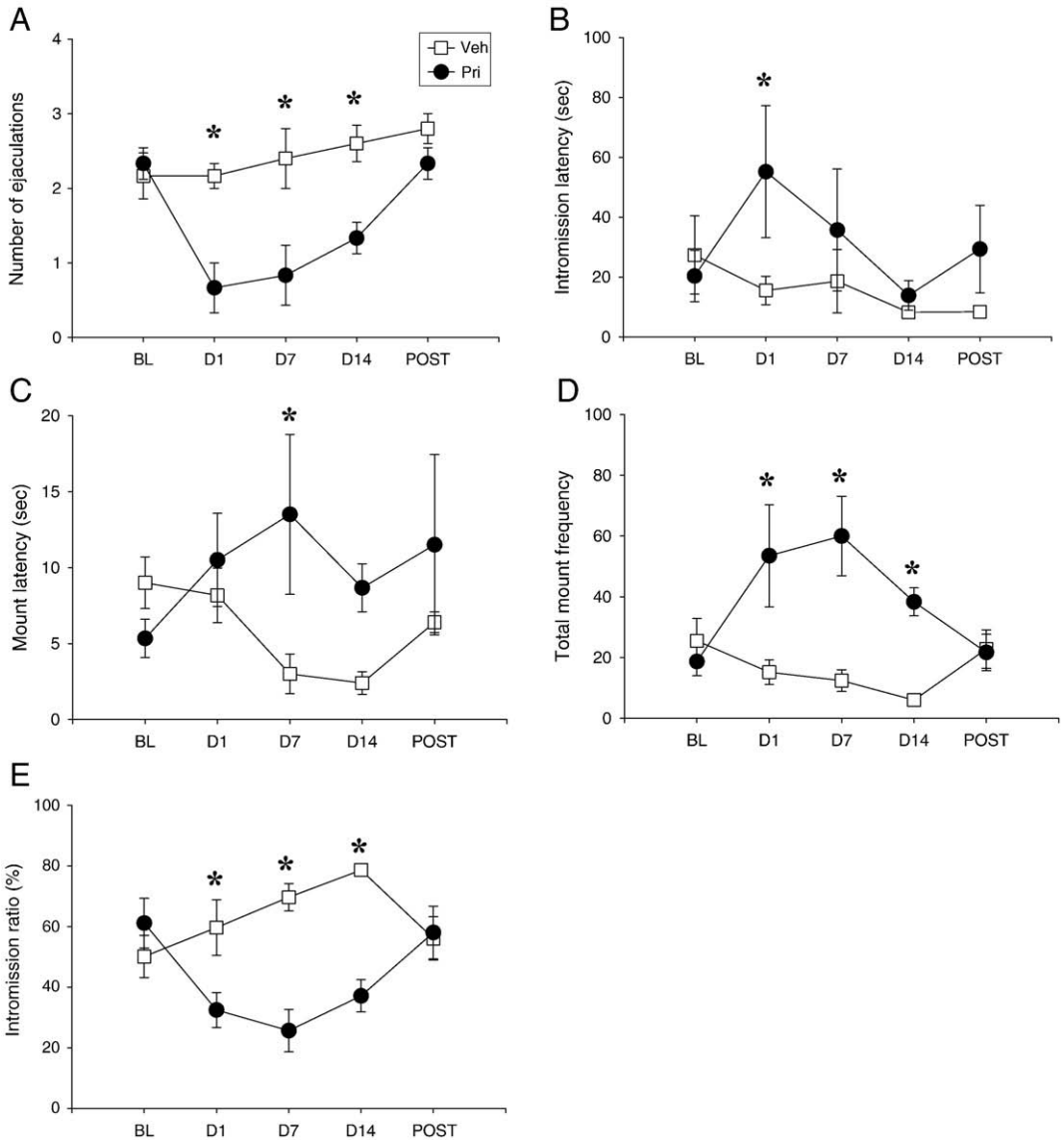

Fig. 1. Primidone impaired male rat sexual behavior. Animals receiving primidone displayed significantly (A) fewer ejaculations, (D) a greater number of mounts, and (E) decreased intromission ratios versus vehicle control animals on test days 1 (D1), 7 (D7), and 14 (D14) but not under baseline conditions (BL) and conditions one week after drug treatment ended (POST). Primidone also increased (B) latency to intromissions on D1 and (C) mounts on D7. Values are expressed as mean ± SEM (*$p<0.05$).

days 1, 7, 14, and post) and a between groups factor of primidone treatment (50 mg/kg or vehicle).

Administration of primidone impaired sexual behavior (see Fig. 1). All terms in the EF analysis were significant ($Fs>3.9$, $ps<0.01$). Post-hoc tests confirmed equivalent EFs during the baseline and post-drug sessions ($Us>7.0$, $ps>0.10$), but significantly lower EFs in the primidone animals compared to vehicle on days 1, 7 and 14 ($Us<25.0$, $ps<0.05$). The primidone-treated rats exhibited longer ILs and MLs than their vehicle-treated counterparts on days 1 and 7 respectively [primidone main effect $F_{(1,9)}=7.0$, $p<0.05$; other $F_{(4,36)}$ s<1.0, $ps>0.40$]. Although there was no main effect of session on MF [$F_{(4,36)}=1.4$, $p>0.20$], there was a significant effect of primidone and a session × primidone interaction ($Fs>4.3$, $ps<0.01$). The groups did not differ from each other during the pre-drug baseline and post-drug washout sessions ($Us>14.0$, $ps>0.80$); however, the primidone-treated rats exhibited significantly higher MF than did the vehicle-treated rats on days 1, 7 and 14 ($Us=0.0$, $ps<0.01$). There were no significant main or interaction effects on IF [session main effect $F_{(4,36)}=2.5$, $ps>0.06$; other $Fs<1.9$, $ps>0.10$]. Given primidone's effects on MF but not IF, IRs also varied significantly by primidone treatment [$F_{(1,9)}=22.9$, $p<0.001$] which interacted with session [$F_{(4,36)}=7.1$, $p<0.001$; main effect of session $F_{(4,36)}=1.2$, $p>0.30$]. Post-hoc tests revealed equivalent IRs during the baseline and post-drug assessments ($Us>11$, $ps>0.50$), but significantly lower IRs in the primidone versus vehicle rats on days 1, 7 and 14 ($Us=0.0$, $ps<0.01$). Ejaculation latency was not analyzed since not all animals receiving primidone displayed ejaculations.

Table 1
Primidone did not affect sexual motivation as measured in an x-maze.

	Baseline	Test day
Vehicle (% choice)	83.3 ± 4.91	90.9 ± 8.03
Primidone (% choice)	93.2 ± 3.07	87.8 ± 3.97
Vehicle (s)	14.4 ± 4.06	10.7 ± 2.20
Primidone (s)	20.9 ± 7.32	11.81 ± 3.25

There were no significant differences between treatment groups in the number of times males chose a goal box containing a female or in the latency to reach the goal box. Values are expressed as mean ± SEM.

Table 2
Primidone did not affect motor activity.

	Day 1	Day 7	Day 14
Vehicle	17.40 ± 1.94	17.60 ± 2.38	21.20 ± 1.53
Primidone	21.00 ± 3.03	15.67 ± 2.12	24.17 ± 1.70

Animals receiving primidone did not differ from vehicle controls in the number of line crosses in their home cage shortly before mating. Line crossings are expressed as mean ± SEM.

3.2. Sexual motivation

Administration of primidone did not affect sexual motivation (see Table 1). Percent choices obtained using the x-maze were analyzed with a 2 × 2 mixed ANOVA with a repeated-measures factor of session (baseline and test) and a between groups factor of primidone treatment (50 mg/kg or vehicle). Analyses revealed no significant main or interaction effects on percent choice of female [$F_{(1,9)} = 0.037$, $p = 0.85$] or latency to reach goal box [$F_{(1,9)} = 0.37$, $p = 0.56$].

3.3. Motor activity

Administration of primidone did not affect motor activity (see Table 2). A 5 × 2 mixed ANOVA yielded a significant main effect of session [$F_{(3,27)} = 3.3$, $p < 0.05$], but no main or interactions effect involving primidone ($Fs < 0.65$, $ps > 0.40$). The only significant change was an overall increase in horizontal movement from test days 7 to 14 ($Z = -2.5$, $p < 0.05$; other absolute $Zs < 1.0$, $ps > 0.30$).

3.4. Sucrose preference

Administration of primidone did not affect sucrose preference (see Fig. 2). Sucrose consumption (ml), water consumption (ml) and relative sucrose preference (sucrose as percent of total consumption) were each analyzed by a 12 × 2 mixed ANOVA with a repeated-measures factor of day (2–6, 8–13) and a between groups factor of primidone treatment. There were no significant main or interaction effects on any of the variables measured across the daily 2-h tests ($Fs < 2.7$, $ps > 0.09$). The mean ± SD sucrose preference over all trials collapsed across primidone groups was 88.7 ± 22.3%.

4. Discussion

Primidone impaired copulation but not motivational aspects of sexual behavior or motor activity, as evidenced by the copulation and x-maze tests. These results partially support the original hypothesis that primidone would affect copulation and sexual motivation. Furthermore, primidone did not affect sucrose consumption, a common measure of general motivation.

Impairments in sexual function were evident throughout the duration of drug administration. Rats treated with primidone displayed fewer ejaculations, required more time to achieve an intromission, and displayed fewer intromissions per attempted mount as evidenced by a lower intromission ratio. These deficits appeared after acute primidone treatment and lasted throughout the chronic treatment phase, suggesting that primidone's effects on sexual function are acute as well as chronic.

The impairments of sexual function displayed by primidone-treated animals were not due to global motor deficits, since both the primidone group and the vehicle group displayed comparable locomotion in the motor tests conducted 15 min post-injection. Although there are several reliable measures of motor activity and ataxia in rodents other than the line-crossing test employed in this study (Crabbe et al., 2005), our focus was sexual behavior, and thus it was important that measures of motor activity be obtained in the mating arena under conditions similar to those present during mating.

Interestingly, the primidone-treated animals displayed a significantly higher number of mounts in all testing sessions. The increased number of mounts likely resulted from an inability to achieve intromissions, which points to erectile dysfunctions. This idea warrants further examination in a future study using more direct measures of erectile activity. The fact that animals receiving primidone continued to mount also suggests that primidone did not impair sexual motivation. Moreover, the ability to mount as seen in animals receiving primidone lends further support to the motor data, since mounts are coordinated motor movements that would be difficult to accomplish under conditions of impaired locomotion.

Copulation tests were terminated on day 14. Although not statistically significant, a trend towards recovery in the number of ejaculations and intromission ratio is observed between days 7 and 14. Additional test sessions may have revealed tolerance to primidone's effects on sexual behavior. Future experiments may use longer treatment to test for possible development of tolerance.

Influences of primidone on sexual motivation were more carefully assessed using an x-maze. No differences were found between the two groups in this test: both the primidone and vehicle groups chose the female on approximately 89% of trials on test day. Additionally, there were no between-group differences in latency to reach the goal box. Again, this indicates that primidone did not impair locomotion and did not impair motivation to copulate with a receptive female. The x-maze data in conjunction with the tests of sexual function suggest that primidone does not impair motivation to copulate, but rather, it impairs the ability to copulate. It is important to note, however, that rats used in the copulation tests were the same used in the x-maze tests. Thus, development of drug tolerance may explain these results. However, the post-chronic-washout test, conducted a week after drug administration was terminated, showed no lingering differences between the two groups in terms of sexual behavior, indicating that all animals returned to baseline prior to x-maze training.

The focus of this study was male sexual function, systematic assessment of primidone's effects on female sexual behavior would also

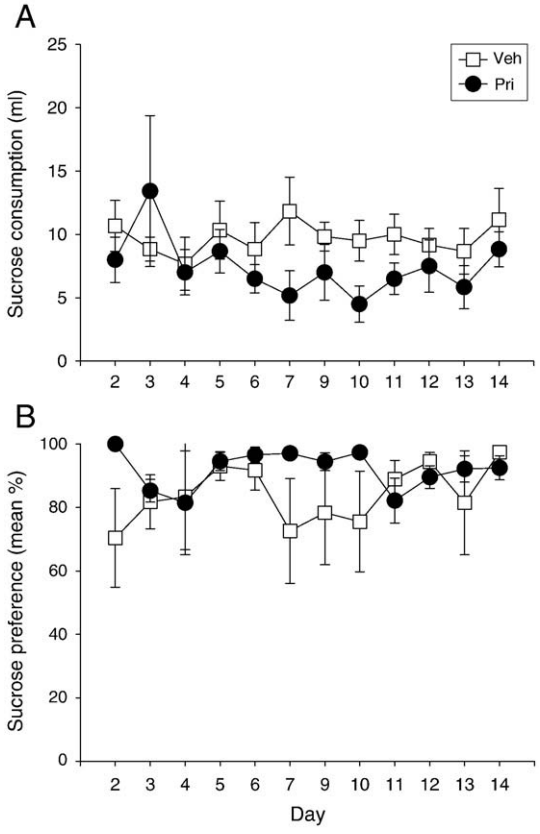

Fig. 2. Primidone did not affect (A) sucrose consumption or (B) preference for sucrose in a sucrose preference test. Values are expressed as mean ± SEM.

be worthy of future inquiry. Also, using neurologically normal rats when assessing influences of an AED on sexual function limits implications of these results for the human condition, since AEDs are normally prescribed to patients suffering from epilepsy. Finally, this study assessed only behavioral effects of primidone administration; it did not examine central mechanisms responsible for the deficits noted here. Studies point to influences by amino acids in the regulation of male sexual behavior (reviewed in Hull et al., 2007; Dominguez, 2009). Since primidone is known to alter GABAergic and glutamatergic activity, it is possible that impairments noted here resulted, at least in part, via changes in these central mechanisms. Male sexual behavior is regulated by activity in several spinal, thalamic, limbic, and mesocorticolimbic structures; amino acids may act in these structures to mediate activity under mating conditions. Future studies will more closely examine influences of primidone on these mechanisms.

Hormone levels were not measured in this study, however it is possible that endocrine factors may have influenced impairments observed here. Human studies show that men with epilepsy often have reduced serum levels of free bioactive testosterone (FT) compared to controls; this decrease in FT may be attributed to an increase in sex-hormone binding globulin (SHBG), which binds to T and decreases its bioavailability (El-Khayat et al., 2003). In a study of adolescent epileptic patients who were being treated with AEDs (carbamazepine, valproate, or phenytoin), El-Khayat et al. (2003) found that testicular volume and penile length were significantly reduced in the epileptic patients receiving treatment. They also found significantly elevated estradiol serum levels in the patients as compared to controls. This effect may be a result of the hepatic enzyme-inducing properties of the three AEDs that were investigated. The induction of hepatic enzymes leads to increased synthesis of aromatase, which converts T to estradiol. Therefore AEDs might not only reduce FT by increasing SHBG, they might also increase the conversion of T to estradiol by aromatase, further decreasing the level of circulating androgens (El-Khayat et al., 2003). The same might apply to primidone, the AED under investigation in the present study, since it too is a potent inducer of hepatic enzyme activity (Perucca et al., 1984).

In summary, primidone impaired sexual function in neurologically normal male rats, but did not influence sexual motivation. These data allow us to begin exploring the physiological, endocrine, and neural mechanisms that underlie AED-induced impairments in sexual function, and whether these mechanisms are different from those underlying sexual motivation.

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