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The Anti-Epileptic Drugs phenytoin and valproate produce reproductive endocrine dysfunction among female rats through its effects on progesterone and its 5 α -reduced metabolites.

By

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ABSTRACT

Background: Many people with seizure disorder experience reproductive endocrine dysfunction (RED). The extent to which RED is due to intrinsic aspects of seizure disorder, and/or therapies used to manage epilepsy, is not well understood. Many anti-epileptic drugs (AEDs), such as phenytoin or valproate, can have effects that alter endocrine status. Phenytoin is a cytochrome p450 enzyme-inducing drug. Valproate is a cytochrome p450 enzyme-reducing AED. **Objective:** To evaluate the effects and mechanism of some AEDs on RED in a rodent model. **Hypothesis:** If AEDs produce RED in part by altering the activities of cytochrome p450 enzymes, then AEDs that disrupt estrous cyclicity and/or sexual behavior will alter levels of steroids, that are products of p450 enzymes, in brain areas mediating such responses.

Methods: Phenytoin (50 mg/kg, IP), valproate (165 mg/kg, IP), or placebo (40% propylene glycol and 10% ethanol in distilled water, IP) was administered to gonadally-intact, female Long-Evans rats, twice daily for four weeks. Estrous cyclicity was monitored daily. After 4 weeks, when rats were in proestrous, they were tested for sexual receptivity. Immediately after completion of sex testing, brain and trunk blood were collected for later radioimmunoassay.

Ovaries and uterus were collected and weighed as a measure of trophic effects. **Results:** Phenytoin and valproate disrupted the estrous cycle and increased aggressive behavior of rats. The levels of 5α -pregnan- 3α -ol-20-one ($3\alpha,5\alpha$ -THP) in the hippocampus and hypothalamus of phenytoin-treated rats were significantly lower than in controls. Levels of progesterone (P_4) in plasma were lower among rats administered valproate compared to the controls. **Conclusion:** AEDs, such as phenytoin and valproate, disrupted estrous cyclicity and sexual behavior of female rats, and reduced levels of hypothalamic, hippocampal, and circulating progestogens, which are products of p450 enzymes.

INTRODUCTION

Epilepsy is a serious neurological disorder affecting over 3 million Americans and over 50 million people worldwide (McHugh and Delanty, 2008). Those who suffer from epilepsy outnumber those suffering from multiple sclerosis, cerebral palsy, muscular dystrophy and Parkinson's disease combined (Meachem, 2009). Furthermore, approximately 200,000 new cases of epilepsy are diagnosed each year, 70% of which appear to be asymptomatic. Epilepsy affects every age group, from infants to the elderly, and can develop at any stage in life. However, in most cases it begins in childhood, at the onset of puberty. On average, a person with epilepsy faces a mortality rate that is 2-3 times greater than that of an unaffected person; (Nouri, 2006). The risk of sudden death among epileptics is 24 times greater than that of the general population (Meachem, 2009). In summary, epilepsy is a pressing problem worldwide.

Epilepsy is associated with Reproductive Endocrine Dysfunction, or RED, in both men and women. Women with epilepsy face a host of challenges, including disruption in reproductive health. Compared to the general population, women with epilepsy have substantially higher chances of developing syndromes associated with infertility, such as polycystic ovary syndrome, hypothalamic amenorrhea, or functional hyperprolactinemia (Bauer and Cooper-Mahkorn, 2008). In a large clinical center 50% of women with epilepsy were found to have menstrual abnormalities, 20% amenorrheic and 30% anovulatory (Engel et al, 2007). Epidemiological studies have shown that 10%-25% of women with epilepsy suffer from Polycystic Ovary Syndrome (**PCOS**) compared with 4-6% of women in the general population (Bauer et al, 2002). A study conducted in the Greater London area showed that women with epilepsy had a significantly reduced birth rate in comparison with women of the general population (Wallace *et al*, 1998). In addition, studies on sexual function in women with epilepsy reported variable

frequencies of sexual dysfunction: 14% in Egypt, 29% in Scandinavia, 36-50% in the United States (Morrell, 1998). This demonstrates that RED is a common problem among women with epilepsy.

It is well established that steroid hormones have great influence on reproductive endocrine function. The central nervous system (CNS) acts both as a target and source of sex steroids and of their metabolites. Neuroactive steroids are steroid hormones that produce functional effects through actions in the CNS. They mediate many diverse neuroendocrine functions (Baulieu, 1997). Among those functions are the release of GnRH, LH, and FSH which are important in ovulation and regulation of the duration of menstrual cycles. Neuroactive steroids also influence female sexual behavior (Frye *et al*, 2006). An important steroid hormone which influences female sexual behavior is Progesterone (P_4), which like all other steroid hormones, is derived from cholesterol. Cholesterol must first be converted to pregnenolone, which when catalyzed by cytochrome P450 forms progesterone. P_4 is the precursor of the mineral corticoid aldosterone which is then reduced to form the many important byproducts such as testosterone, estradiol, and the metabolite of P_4 , 5 alpha-pregnane-3,20-dione (5α -DHP), which is further broken down into 5alpha-pregnan-3alpha-ol-20-one ($3\alpha,5\alpha$ -THP) (Frye and Paris, 2008). To gain insights into the nature of the effects of steroid hormones on reproductive endocrine function and sexual behavior, a rat model is used in this study.

In rodent models, sexual activity is dependent on a modulator of lordosis (the stereotypical posture that female rodents exhibit in response to male-typical stimuli), and the control of behavioral processes such as aggression and anxiety (Frye and Rhodes, 2008). $3\alpha,5\alpha$ -THP, a metabolite of P_4 , has been shown to mediate such behaviors. Rats have elevated $3\alpha,5\alpha$ -THP in behavioral estrus, a period during which they are most receptive to mating and demonstrate more exploration, anti-anxiety, and pro-social behaviors than rats in diestrus, a

period in which they are least receptive to mating (Frye, Paris, and Rhodes 2008). Elevated $3\alpha,5\alpha$ -THP levels, independent of P_4 , also increase hopping and darting behavior (proceptivity) that female rats make toward male rats to achieve sexual contacts. Overall, the metabolism and presence of $3\alpha,5\alpha$ -THP is a critical factor in the success of modulating rodent mating.

$3\alpha,5\alpha$ -THP has multiple targets in the brain to modulate its effects on sexual behavior. In the ventral tegmental area (VTA) of the midbrain, $3\alpha,5\alpha$ -THP works to control the consummatory aspects of sexual behavior; this includes stimulating and controlling lordosis. Decreased $3\alpha,5\alpha$ -THP concentrations in the midbrain region significantly decrease lordosis responses (Frye et al., 2008). The $3\alpha,5\alpha$ -THP concentrations at GABAergic substrates in the hippocampus and hypothalamus work to mediate motivational and anxiety aspects of sexual behavior. When there are increased levels of $3\alpha,5\alpha$ -THP in the hippocampus, female rats demonstrate less species-typical anxiety-like behavior and conspecific-avoidance, spending more time in affiliation with their mates (Frye and Rhodes, 2008). Thus, the role of $3\alpha,5\alpha$ -THP in the different parts of the rodent's brain dramatically affect mating behavior.

Anti epileptic drugs (AEDs) have the potential to affect brain function and, more specifically, the production and metabolism of steroid hormones, like $3\alpha,5\alpha$ -THP, which affect sexual behavior. Studies done on women taking AEDs show that the occurrence of RED is significantly higher in those that take cytochrome p450 enzyme-inducing AEDs, such as phenytoin, than those that do not (Sheth and Montouris, 2008). AEDs which induce cytochrome p450 increase the metabolism of gonadal and adrenal steroid hormones which induce the synthesis of sex hormone-binding globulin. As the potential for protein binding ability increases, the biologically active fraction of hormone levels in circulation decreases. This reduction in sex steroid hormones is commonly associated with sexual dysfunction (Morrell, 2005). Prior studies have demonstrated that serum concentrations of estrogens and androgens are indeed reduced in

women with epilepsy receiving enzyme inducing AEDs (Morrell, 2005). This, in turn, is associated with reproductive endocrine dysfunction such as polycystic ovaries and hypogonadotropic hypogonadism (Macphee *et. al.*, 1998; Stoffel-Wagner *et. al.*, 1998). Similar to cytochrome p450 enzyme inducing AEDs, cytochrome p450 enzyme-reducing AEDs, such as valproate, have marked effects on steroid hormone levels. Enzyme reducing AEDs are progesterone antagonists and they dramatically increase the presence of circulating androgens (Isojarivi *et. al.*, 2004). This consequently disrupts the ratio of androgens to estradiol, causing reproductive problems such as polycystic ovaries (Morrell, 2003). The critical ratio of androgens to estradiol is in other words the ratio between female and male sex steroid hormones. The most common type of RED experienced with valproate users is anovulatory cycles (Morrell, 2004). Overall, the AEDs, phenytoin and valproate, which have been shown to alter steroid hormone levels in the brain, valproate reducing the amount of estradiol and progesterone in circulation and phenytoin reducing the metabolically active levels of steroid hormones, can cause many undesirable and serious side effects.

Studies such as this one are critical in understanding and evaluating the effects and mechanism of some AEDs on RED. It is difficult, however, to determine whether RED is a direct result of epilepsy or the AED treatment used to combat the disorder. The rat model is helpful in isolating the effects of the AEDs on steroid hormone functions as rats are epilepsy free. Subjecting human beings to similar epilepsy experimentation presents ethical dilemmas as it is simply not feasible to withhold treatment from a control group due to the devastating consequences of epileptic seizures which may include brain damage or death. The data suggests phenytoin, a cytochrome p450 enzyme inducing drug, and valproate, a cytochrome p450 enzyme reducing drug, could have marked effects on steroid hormone levels. To determine if this is the

case, we correlated steroid levels in the brain areas controlling estrous cyclicity and sexual behavior in rats treated with AEDs that potentially produce RED in humans.

MATERIALS & METHODS

These methods were pre-approved by the Institutional Animal Care and Use Committee at The University at Albany-SUNY.

Subjects

Female, intact rats (N=38) were approximately 50 days old and were acquired from our breeding colony at The University at Albany-SUNY. Rats were housed in polycarbonate cages (3-4 rats per cage), in a temperature- (21 ± 1 °C) and humidity- (50 ± 5 %) controlled room in our Laboratory Animal Care Facility. Rats were maintained on a reversed 12 hr light-dark cycle (lights off at 0800 h) and had *ad libitum* access to food and water throughout the study.

Determination of Estrous Cycle Phase

Vaginal epithelium was obtained between 0800-0900 h via lavage and examined microscopically daily to determine the day of the estrous cycle. Cycle phase was determined per previous methods (Frye, 2001; Marcondes et al., 2002). Briefly, the proestrous phase, the time when endogenous estrogens and progestogens peak, was characterized by the presence of many nucleated epithelial cells. The estrous phase, when estrogens are low and progestogens are declining was distinguished by cornification of the vaginal epithelium. Cornification is the stratification of epidermal tissues and the process of forming an epidermal barrier on the cells coupled with the production of keratin (Lippens, 2005). It is characterized by cells with very rugged edges and the loss of nuclei and organelles. The diestrous phase, when estrogens and progestogens are at nadir, was characterized by the lack of epithelial cells and the presence of leukocytic cells. Rats were cycled through two estrous cycles (demonstrating proestrous once

every 4-5 days) prior to manipulation in order to verify typical estrous cyclicity. Only rats that demonstrated 4-5 day cycles before manipulation were utilized in the study.

Preparation of AED Regimen

AEDs were purchased from Sigma-Aldrich (St. Louis, MO). Phenytoin was dissolved in a propylene glycol vehicle (40% propylene glycol and 10% ethanol in distilled water) and valproate was dissolved in 0.9% sodium chloride.

Procedure

Rats were randomly assigned to receive twice daily, intraperitoneal (IP) injections of phenytoin (50 mg/kg, IP), valproate (165 mg/kg, IP), or a commensurate volume of vehicle (40% propylene glycol and 10% ethanol in distilled water, IP) at 11:00 h and 15:00 h for at least 28 days. During this time, vaginal cytology was recorded daily. After 28 days of treatment, rats were tested in the mating paradigm described below at their next sexually-receptive (proestrous) phase of their estrous cycle. The rats continued to receive AED treatment until sex-testing. Because of the need to test rats when sexually-receptive, there were differences in the length of treatment among some rats (the treatment range was 28-33 days) but these differences did not account for a significant amount of variance on any measure of parameter. Immediately following completion of sex-testing; brain, ovaries, uterus, and serum were collected and wet weights were documented. All tissues were stored at -80 °C until the radioimmunoassay was performed.

Sex Testing

Sex testing was conducted in a chamber 37.5 x 75 x 30 cm as previously reported (Hardy & DeBold, 1972; Frye *et al.* 2000). Sexually-vigorous male studs were briefly paired with sexually-receptive stimulus females in order to ensure immediate mounting. Each experimental

female rat was placed in the chamber with a stimulated male and observed until either ten mounts were achieved, until male ejaculation, or for ten minutes if neither ten mounts nor ejaculation were achieved. Behavior was observed by two investigators, and mean scores were utilized in all behavioral analyses. The frequency and intensity of lordosis (quantified by rating of dorsiflexion on a scale of 0–3) in response to male mounting was recorded (Hardy & DeBold, 1972). In addition, the frequency of proceptive (hopping, darting, ear-wiggling) and aggressive (vocalizations, defensive posture) behaviors were recorded as described (Frye et al 2001).

Tissue Preparation

Brains were kept on ice and the midbrain, hippocampus, hypothalamus, and cortex were removed, and the remaining tissue without the cerebellum was used as a control (“inter-brain”) (Frye and Rhodes, 2006ab). Following the extractions of the separate brain regions, steroids were extracted from brain tissue as described below.

Radioimmunoassay for Steroid Hormones

P₄, 5 alpha-pregnane-3,20-dione (5 α -DHP), 3 α ,5 α -THP, and Androstane-3 α -Diol-G (3 α -diol) concentrations were measured using the previously reported methods described below (Choi and Dallman, 1999; Frye and Bayon, 1999; Frye et al., 1996). Following tissue collection, some brains were frozen on dry ice, blood was centrifuged, the serum saved, and tissues were stored at -80 °C for later analysis.

Radioactive Probes

The radioactive probes used for the assays were as follows: [³H] P₄ (catalog number NET-208) with a specific activity of 47.5 Ci/mmol; [³H]3 α ,5 α -THP (catalog number NET-1047) with a specific activity of 65.0 Ci/mmol; 5 α -DHP catalog number (NET-1047) with a specific activity of 65.0 Ci/mmol, 3 α -diol (catalog number NET-806) with a specific activity of 41.00 Ci/mmol were purchased from Perkin Elmer (Boston, MA).

Extraction of Steroids from Serum

P₄, 5 α -DHP, 3 α ,5 α -THP, and 3 α -diol were extracted from serum with ether following incubation with water and 800 cpm of ³H steroid (Frye and Bayon, 1999). Test tubes containing steroid and ether were evaporated to dryness in a speed vacuum. Dried down samples were reconstituted with pH 7.2 phosphate assay buffer to the original serum volume.

Extraction of Steroids from Brain Tissues

P₄, 5 α -DHP, 3 α ,5 α -THP, and 3 α -diol were extracted from brain tissues following homogenization with a glass/glass homogenizer in 50% methanol, 1% acetic acid. Tissues were centrifuged at 3,000 x g and the supernatant was chromatographed on Sepak-cartridges. Steroids were washed with 50 % methanol then eluted with 100% methanol. Solvents were removed using a speed vacuum. The samples were reconstituted in 500 μ l of assay buffer.

Antibodies

The P₄ antibody (P#337 from Dr. G.D. Niswender, Colorado State University) was diluted 1:30,000 and typically binds between 30% and 50% of [³H]P₄. The 5 α -DHP (X-947) and 3 α ,5 α -THP antibodies (#921412-5, purchased from Dr. Robert Purdy, Veterans Medical Affairs, La Jolla, CA) was diluted 1:5000 and binds between 40-60% of [³H]3 α ,5 α -THP. The 3 α -diol antibody (X-144; Dr. P.N. Rao, Southwest Foundation for Biomedical Research, San Antonio, TX) was diluted 1:20,000 and binds approximately 96% of [³H]3 α -diol .

Set-up and Incubation of Radioimmunoassays

The range of the standard curves were 0-8000 pg for P₄, 5 α -DHP, and 3 α ,5 α -THP, and 0-2000 pg for 3 α -diol. Standards were added to assay buffer followed by addition of the diluted antibody and [³H] steroid. Total assay volumes were 800 μ l for P₄, 900 μ l for 3 α -diol, 950 μ l for 5 α -DHP, and 1250 μ l for 3 α ,5 α -THP. All assays were incubated overnight at 4°C.

Termination of Binding

Extraction of the free steroids was accomplished by the rapid addition of dextran-coated charcoal. Following incubation with charcoal, samples were centrifuged at $3000 \times g$ and the supernatant was pipetted into a glass scintillation vial with 5 ml scintillation cocktail. Sample tube concentrations were calculated using the logit-log method of Rodbard and Hutt (1974), interpolation of the standards, and correction for recovery with Assay Zap. The logit-log method is an empirical technique to convert the sigmoid calibration curve into a reasonably straight line, so that a straight line approximation is acceptable. The inter- and intra-assay reliability coefficients were: for P_4 , 0.11 and 0.04, for 5α -DHP, 0.11 and 0.04, for $3\alpha,5\alpha$ -THP 0.09 and 0.04, and for 3α -diol 0.09 and 0.04.

Statistical analyses

The significance of differences in estrous cyclicity were determined via chi-square analysis. Main effects on tissue weights, neuroendocrine, and behavioral measures were evaluated by separate one-way analyses of variance (ANOVAs) with the AED treatment condition (vehicle, phenytoin, valproate) as the independent variable. Differences between groups were determined via Fisher's protected least significant differences (PLSD) post-hoc tests. The alpha level for statistical significance was p less than 0.05. Trends were reported when p was less than 0.10.

RESULTS

Estrous Cyclicity and Uterine/Ovarian Weight

AED treatment significantly altered estrous cyclicity among female rats [$\chi^2(2, N = 38) = 5.67, p < 0.05$]. Over the 4 week study, an average of 8 ± 2 cycles was observed. Of the thirteen females administered phenytoin, and the thirteen females administered valproate, only two from each group demonstrated a normal 4-5 day cycle following treatment. Of the twelve female controls administered vehicle, nine demonstrated normal 4-5 day cycles (*Figure 1*). Compared to 75 % of vehicle rats which cycled properly, only 15% of phenytoin ($p < .05$) and 15% valproate

rats ($p < .05$) cycled properly (*Figure 1*). Neither uterine nor ovarian weight was significantly affected by phenytoin or valproate treatment compared to the control.

Neuroendocrine Endpoints

AED treatment significantly altered CNS steroid concentrations in some regions but not in others. First we look at areas related to the control of sexual behaviors: the hippocampus, midbrain, and hypothalamus. In the hippocampus, $3\alpha,5\alpha$ -THP levels were significantly lower in phenytoin-administered rats in comparison to valproate and/or vehicle groups ($p < .05$; *Figure 2* and *Table 2*). Phenytoin rats also exhibited a trend of increased levels of P_4 in the hippocampus compared to the vehicle group ($p < .10$; *Table 2*). There were no other differences in steroid hormone levels of 5α -DHP or 3α -diol in the hippocampus.

In the hypothalamus, phenytoin rats exhibited a trend of lower $3\alpha,5\alpha$ -THP levels ($p < .10$; *Table 2*) compared to the vehicle group. There were no other differences in steroid hormone levels of P_4 , 5α -DHP, and 3α -diol in the hypothalamus. There was also, no differences of steroid hormone levels found in the Midbrain. Overall, in areas controlling sexual behavior there was only altered steroid hormone differences in the hippocampus and hypothalamus, but not the midbrain.

As a control measure, there was no difference between the phenytoin group and the vehicle group in concentrations of P_4 , 5α -DHP, $3\alpha,5\alpha$ -THP and 3α -diol in the cortex, and interbrain regions (*Table 2*). Phenytoin rats also exhibited no differences among serum concentration as compared to the Vehicle group (*Table 2*). Although differences in steroid hormone levels for phenytoin rats were observed in the hippocampus and hypothalamus, there was no difference compared to the vehicle group in the midbrain, cortex, and serum.

Valproate rats showed a significant decrease in serum P₄ and a significant increase in serum 5 α -DHP concentrations ($p < .05$; *Table 2*). The valproate group exhibited no difference in comparison to the vehicle group in concentrations of P₄, 5 α -DHP, and 3 α ,5 α -THP and 3 α -diol in all 5 brain regions. Overall, valproate had no effect on steroid hormone levels in the brain, but significantly altered their concentration in the blood (*Table 2*).

Behavioral Endpoints

Phenytoin rats exhibited a significantly higher aggression quotient (aq) and lower proceptivity quotient (pq) than the vehicle rats ($p < .05$) (*Figure 3*). However, phenytoin rats showed no main effect for lordosis quotient (lq), lordosis rating (lr), mount latency or total sex duration (*Table 1*).

Valproate rats showed a tendency to be more aggressive and less proceptive during sex testing (*Figure 3*). Although valproate rats exhibited higher aggression and were less proceptive during sex testing, there was no main effect for lq, lr, mount latency or total sex duration (*Table 1*).

DISCUSSION

Our hypothesis which stated that the anti-epileptic drug phenytoin would produce reproductive endocrine dysfunction among female rats was supported. Eleven out of the thirteen rats injected with phenytoin exhibited abnormal cycling compared to only three out of the eleven rats in the control group that showed abnormal cycling. In addition, the study showed a significant increase in aggression and a tendency to exhibit decreased proceptivity during standard sex testing in rats treated with phenytoin. The control group of rats, treated with vehicle, exhibited no increased aggression or decreased proceptivity or abnormal cycling in our study. Increased aggression, and decreased proceptivity are indications of reproductive endocrine dysfunction (Morrell, 2003).

Our hypothesis further stated that the anti-epileptic drug phenytoin would produce reproductive endocrine dysfunction among female rats through its effects on progesterone and its 5α -reduced metabolites. Evaluation of hormone dynamics in the brain for the phenytoin rats showed significantly lowered $3\alpha,5\alpha$ -THP levels in the hippocampus and hypothalamus. The significant decrease in the $3\alpha,5\alpha$ -THP levels in the hippocampus correlates strongly to the marked increase in aggressive and anti social behavior of female rats during mating. This data correlated with our predictions and further supported our hypothesis.

These results are also consistent with previous studies. The data from this study demonstrate that AEDs phenytoin significantly disrupt estrous cyclicity which is consistent with the findings of Martha J. Morrell (Morell 2003; Morrell et al, 2003). To cycle properly it is essential to have proper amounts of free steroid hormones in circulation (Frye, 2008). Enzyme inducing AEDs reduce the levels of free steroid hormones in circulation due to the effect of globulin-binding proteins (Morrell, 2005).

Our hypothesis which stated that the anti-epileptic drug valproate would produce reproductive endocrine dysfunction among female rats was supported. Eleven out of the thirteen rats injected with valproate exhibited abnormal cycling compared to only three out of the eleven rats in the control group that showed abnormal cycling. In addition, rats treated with Valproate showed decreased proceptivity. The control group of rats, treated with vehicle, exhibited no decreased proceptivity and little abnormal cycling in our study. Abnormal cycling and decreased proceptivity are indications of reproductive endocrine dysfunction (Morrell, 2005).

The data from this study demonstrate that the AED valproate significantly disrupts estrous cyclicity which is consistent with previous studies such as the findings of Martha J. Morrell (Morell 2003; Morrell et al, 2003). To cycle properly it is essential to have normal levels of both estrogen and progesterone (Morrell, 2003). We predicted that valproate would produce

reproductive endocrine dysfunction among female rats through its effects on progesterone and its 5α -reduced metabolites. Our study shows that valproate significantly reduced the levels of P_4 in serum. This disruption in the P_4 concentrations caused a higher gonadal and adrenal androgen to progesterone ratio which correlates closely with ovulatory dysfunction (Morrell and Montouris, 2004). This is consistent with the fact that valproate is documented to have strong effects on menstrual cycles.

In addition, our study extends the current literature as it examined the dynamics of how AEDs produce REDs by closely monitoring the role of steroid hormones in the mid brain region, the hippocampus and hypothalamus in relation to normal sexual activity. The rats treated with phenytoin exhibited no signs of consummatory dysfunction, but showed significant motivational dysfunction in the form of increased aggression and antisocial behavior during mating. Evaluation of hormone levels in the brain for these rats showed significantly lowered $3\alpha,5\alpha$ -THP levels in the hippocampus and hypothalamus, and minimal effects on $3\alpha,5\alpha$ -THP levels in the midbrain. The significant decrease in the $3\alpha,5\alpha$ -THP levels in the hippocampus correlates strongly to the marked increase in aggressive and anti social behavior of female rats during mating. The minimal effects on $3\alpha,5\alpha$ -THP levels in the midbrain correlates to the little, if any, effect on the lordosis ratings and quotients of the rats in the study.

Future Direction

In the future, there are plans to repeat the same study with the addition of another AED, lamotrogene, which is believed to have minimal, if any, effect on steroid hormones. The group receiving lamotrogene will serve as the negative control since this specific AED is not supposed to affect the hormone levels and thus should not produce the side effect of sexual dysfunction. Comparing the results of the rats receiving phenytoin and valproate treatment with the negative

control group will help us understand the effects of the treatment on sexual dysfunction in rats more accurately. This will show whether the sexual dysfunction experienced is primarily due to phenytoin and valproate's effects on steroid hormones as opposed to other mechanisms.

CONCLUSION

Using a rat model made it possible to isolate the effects of phenytoin and valproate treatments on RED independent of epilepsy. The results of the study clearly show that both phenytoin and valproate disrupt the estrous cycle and increase reproductive endocrine dysfunction among female rats. In addition, this study indicates that phenytoin significantly decreases $3\alpha,5\alpha$ -THP levels in the hippocampus, while having no effect on the concentrations of steroid hormones in the midbrain.

Anti-epileptic drugs have worked wonders for epileptic patients, but it is essential to re-examine their widespread use for other disorders. AEDs are often used in treating a variety of health problems from bi-polar disorder to congenital heart defects to chronic pain. Gaining a better and a more accurate understanding of the potential side effects of AEDs will help physicians weigh the need for AED treatment against the side effects when considering the drugs. Research studies such as this one will promote such understanding and may provide helpful insights into the development of improved AED treatments.

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FIGURE LEGENDS

Figure 1 Differences in Cyclicity. Percent cycling was determined by dividing the number of proper 4-5 day estrous cycles in assigned group divided by total number of cycles observed
(* Denotes a significant difference $p < .05$), (# Denotes a $p < .10$)

Figure 2 Differences in $3\alpha,5\alpha$ -THP, and 3α -diol steroid hormone levels in the hippocampus. Concentrations were calculated using the logit-log method of Rodbard and Hutt (1974), interpolation of the standards, and correction for recovery with Assay Zap.
(* Denotes a significant difference $p < .05$), (# Denotes a $p < .10$)

Figure 3 Differences in Proceptivity and Aggressive behavior. Proceptivity quotient was determined by number of mounts with female displaying proceptive behavior divided by number of mounts. Aggressive quotient was determined by number of mounts followed by aggressive behavior divided by total amount of mounts.
(* Denotes a significant difference $p < .05$), (# Denotes a $p < .10$)

Table 1: Standard Sex testing measures. Lordosis quotient was determined by number of mounts with female displaying lordosis divided by total amount of mounts. Lordosis rating was determined by the sum of the lordosis ratings divided by the total number of rats. Mount latency was found by time required for male to attempt the first mount.
(* Denotes a significant difference $p < .05$), (# Denotes a $p < .10$)

Table 2: Endocrine Data. Concentrations were calculated using the logit-log method of Rodbard and Hutt (1974), interpolation of the standards, and correction for recovery with Assay Zap.
(* Denotes a significant difference $p < .05$), (# Denotes a $p < .10$)

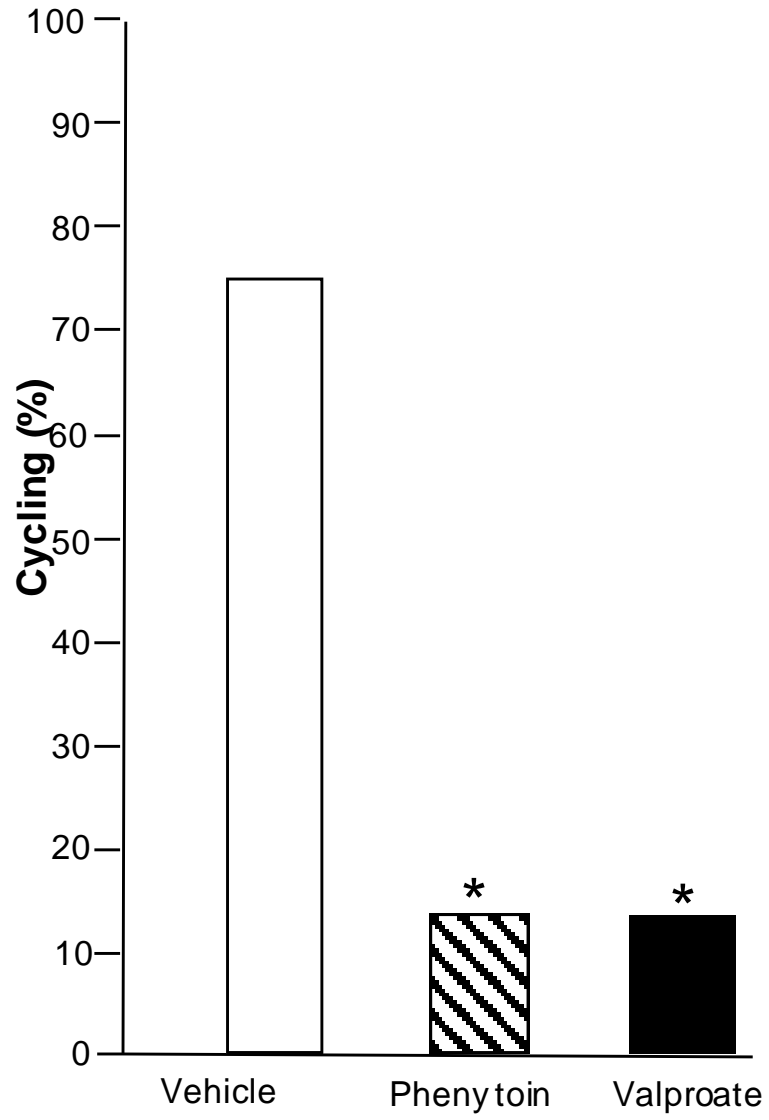


Figure 1

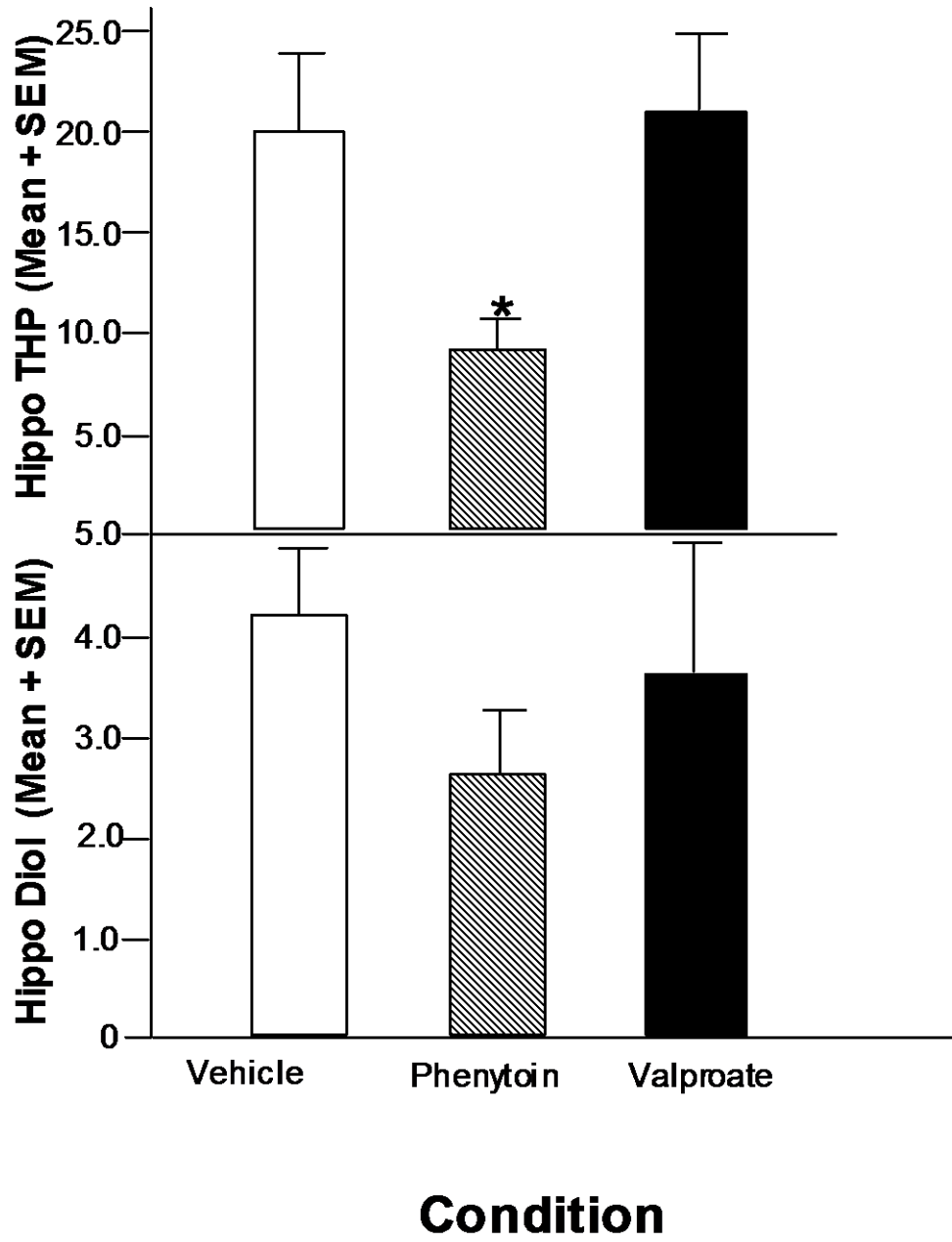


Figure 2

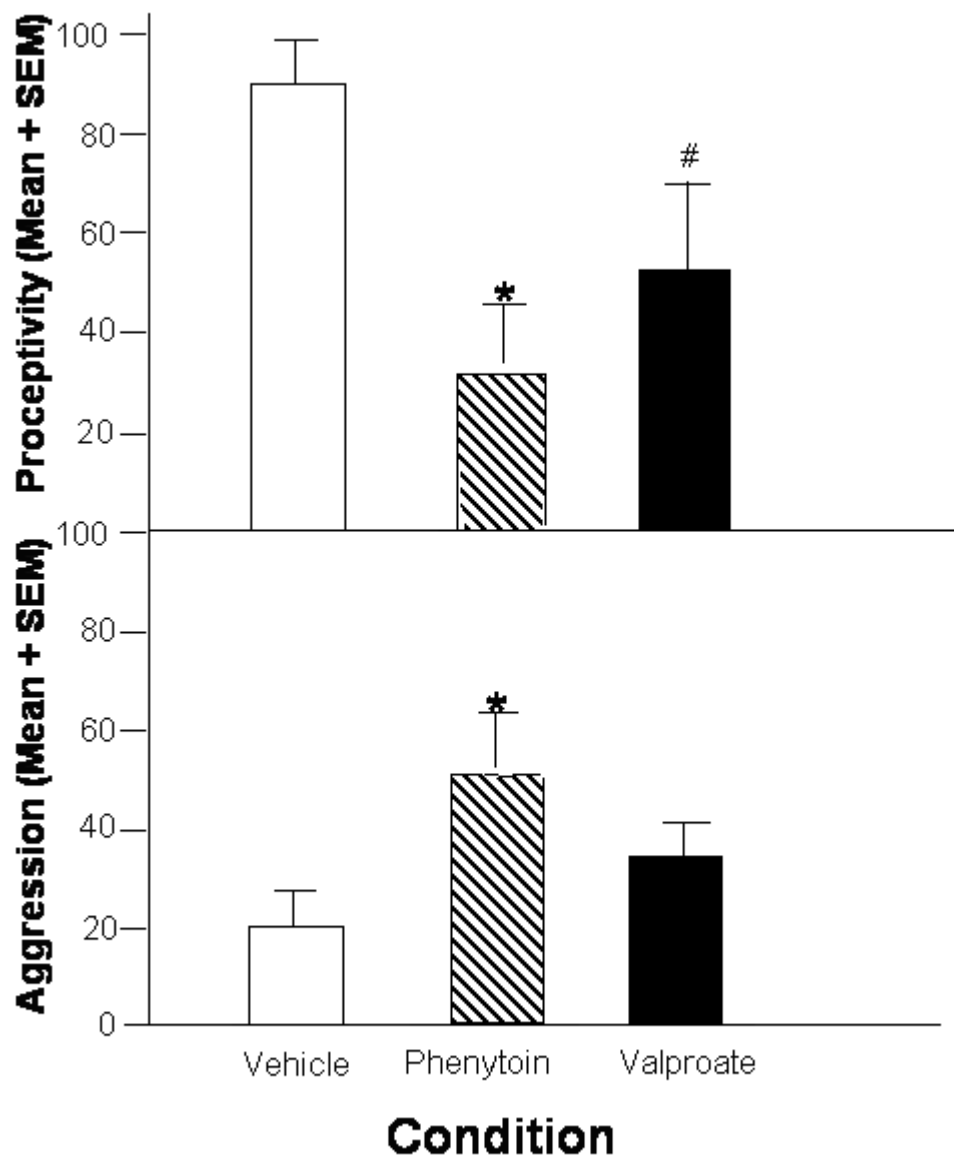


Figure 3

	Vehicle	Phenytoin	Valproate
Lordosis quotient (%)	84.2 ± 9	89.3 ± 7	85.8 ± 10
Lordosis Rating (1-4)	1.9 ± 0.2	2.0 ± 0.3	1.7 ± .25
Sex Total Duration (s)	196.3 ± 38	146.2 ± 36	170 ± 46
Mount Latency (s)	33.6 ± 15	39.5 ± 10	32.5 ± 10

Table 1

	Saline	Phenytoin	Valproate
Midbrain			
P ₄	5.6 ± 2.5	3.5 ± .7	5.2 ± 2.1
5α-DHP	38.9 ± 1.2	61.9 ± 18.7	68.6 ± 19.8
3α,5α-THP	12.7 ± 3.9	13.6 ± 5.2	8.0 ± 2.6
3α-diol	7.1 ± 1.24	8.1 ± 2.5	6.5 ± 1.3
Hypothalamus			
P ₄	6.5 ± 2.0	4.3 ± 1.3	6.8 ± 2.2
5α-DHP	69.1 ± 6.2	51.0 ± 12.4	35.5 ± 15.3
3α,5α-THP	57.1 ± 16.7	28.0 ± 9.0 #	31.7 ± 8.7
3α-diol	16.7 ± 4.9	20.9 ± 4.9	20.2 ± 4.2
Hippocampus			
P ₄	7.4 ± 1.8	15.9 ± 4.1 #	11.6 ± 3
5α-DHP	26.8 ± 9.5	31.0 ± 7.3	14.2 ± 4.4
3α,5α-THP	19.8 ± 4.6	8.6 ± 2.3	22.1 ± 4.2
3α-diol	4.2 ± 0.6	2.3 ± 0.5	3.7 ± 1.3
Cortex			
P ₄	14.9 ± 3	15.6 ± 2	18.4 ± 2.7
5α-DHP	13.4 ± 3.7	10.8 ± 4.1	16.9 ± 4.1
3α,5α-THP	15.6 ± 5.1	15.2 ± 5.1	8.9 ± 3.7
3α-diol	4.7 ± 1.7	3.1 ± .4	4.6 ± 1.4
InterBrain			
P ₄	1.7 ± .5	1.0 ± .32	2.2 ± .76
5α-DHP	39.7 ± 9.7	43.1 ± 12.6	28.2 ± 10.0
3α,5α-THP	13.7 ± 4.1	15.2 ± 1.5	17.1 ± 5.7
3α-diol	4.9 ± 1.1	4.9 ± 1.8	3.3 ± 1.0
Serum			
P ₄	25.0 ± 6.2	19.3 ± 4.5	12.4 ± 3.1 *
5α-DHP	23.8 ± 12.4	36.2 ± 9.7	72.7 ± 14.0 *
3α,5α-THP	54.2 ± 11.5	49.6 ± 12.4	29.8 ± 10.1 #
3α-diol	24.6 ± 4.7	18.7 ± 2.4	21.8 ± 2.5

Table 2