Drugs - Real World Outcomes
https://doi.org/10.1007/s40801-025-00503-z

ORIGINAL RESEARCH ARTICLE

Evidence of Sex-Related Pharmacodynamic Differences in Photosensitive Epilepsy Treated with Valproate: Findings from a Retrospective, Observational, Single-Center, Within-Patient, Cohort Study

Dorothee Kasteleijn-Nolst Trenité[1,2] · Ronald C. Reed[3] · Alessandro Ferretti[4] · Anteo Di Napoli[5] · Pasquale Parisi[4]

Accepted: 18 May 2025

Abstract

Background and Objective Sexual dimorphism in drug efficacy, beyond pharmacokinetics (PK), remains underexplored. Significant sex differences exist in drug metabolism and adverse events, highlighting the need for personalized medicine. The objective of our study was to assess whether there are sex differences in the pharmacodynamic (PD) response to valproic acid (VPA) in photosensitive epilepsy, focusing on electroencephalographic (EEG) biomarkers (e.g., photoparoxysmal response [PPR] raw data and transformed PPR data, the standardized photosensitivity range [SPR]) that cannot be attributed to pharmacokinetics alone. On the basis of some exploratory published evidence plus our own clinical observations of VPA treatment in patients with epilepsy plus photosensitivity over time, we hypothesized that an EEG pharmacodynamic difference might exist between females and males.

Methods We conducted a retrospective, observational, single-center, within-patient EEG cohort study conducted on antiseizure medicine (ASM)-naïve photosensitive individuals before and after VPA treatment (nonrandomized). The data we reviewed had been collected from a referral hospital in the Netherlands from 1990 to 2000. Changes in EEG data, including raw PPR data (transformed into SPR), were analyzed before and after VPA therapy in 48 patients, including 27 females and 21 males, ranging in age from 8 to 50 years old for the entire cohort. Co-primary outcomes included a between-sex comparison in the distribution of within-patient SPR changes from pre-VPA to steady-state VPA therapy, and complete PPR elimination on EEG. Secondary outcomes included the comparison of percentage of males and females meaningfully responding to VPA across SPR change categories, VPA dose, potential impact of plasma [VPA] concentrations on SPR changes, and associaton of patient age with SPR values. Statistical analyses included univariate linear regression models, chi-squared tests, non-parametric Wilcoxon–Mann–Whitney tests, and Fisher's exact tests.

Results Our first co-primary outcome revealed a statistically significant difference in the distribution of within-patient SPR changes from pre-VPA to steady-state VPA therapy. Males experienced a significantly greater reduction in SPR compared with females. The mean decrease in SPR was -7.0 ± 2.6 in males only versus -3.9 ± 3.3 in females only ($p = 0.0018$). The next co-primary outcome, the percent of patients with complete PPR elimination, or a SPR value = 0 on second EEG, was observed in ten (47.6%) males compared with four (14.8%) females, a 3.2-fold difference ($p = 0.0237$). One secondary outcome, the percentage of males with a VPA clinically meaningful to optimal response was 1.93-fold greater than females, at 100:51.8%, respectively ($p < 0.0001$). Between-sex VPA total daily milligram dose did not differ. Plasma [VPA] concentrations, although nearly twice as high in females, were not statistically different between sexes and [VPA] did not correlate with SPR changes in females.

Conclusions In our mixed-age population of pediatric, adolescent, and younger adults, significant sex differences were observed in the response of the PD EEG biomarkers, the PPR and SPR, to VPA for both primary outcomes. Males showed both a statistically greater reduction in EEG SPR and a statistically higher percent elimination of PPR on EEG with chronic VPA treatment compared with females. The percent of males having a clinically meaningful EEG SPR response to VPA, a secondary outcome, was almost two-fold greater for males compared with females. These findings emphasize the importance of considering sex in treatment planning and suggest that inherent biological differences may influence drug efficacy, advocating for further research to enhance personalized medical strategies.

Extended author information available on the last page of the article

Published online: 28 June 2025

Key Points

Sex differences exist in pharmacokinetics and adverse effects for drugs in general, including antiseizure medicines (ASMs), but little information exists for sex differences in ASM efficacy.

Our retrospective analysis of two sequential electroencephalograms (EEGs) within-patients with epilepsy plus photosensitivity analyzed an EEG biomarker of clinical seizures, the photoparoxysmal response (PPR, transformed into the standardized photosensitivity range [SPR]), before and after steady-state treatment with just one ASM—valproic acid (VPA).

Males responded better to VPA than females—all 21 males had a clinically meaningful EEG-SPR response to VPA compared with half that among 27 females, despite higher plasma [VPA] concentrations in females. In addition, 3.2 times as many males exhibited PPR elimination compared with females.

1 Introduction

Recognition of between-sex differences in drug absorption, metabolism/elimination, protein binding, rates of drug-induced adverse events (AEs), and drug pharmacodynamics is crucial for healthcare practitioners aiming to achieve individualized drug regimens or "personalized medicine." Inherent between-sex differences in pharmacokinetics (PK) for several common drugs have been identified and published as comprehensive reviews [1–6], including drug bioavailability/bioequivalence [7]. These differences are not solely due to pregnancy- or menstrual cycle-related drug kinetic changes [8]. In particular, the PK of certain antiseizure medications (ASMs), such as carbamazepine [9], pregabalin [10], and valproic acid (VPA) [11–13], have been found to differ between males and females. Between-sex differences in drug-induced AEs are common. A systematic analysis revealed sex differences in AEs for 46% of the top 20 common drug-combination treatment regimens [14]. Females have a twofold greater risk than males of experiencing drug-induced AEs resulting in hospitalization [15]. In England, young females experience AEs to newly marketed drugs 60% more often [16]; this pattern has been observed across multiple countries [17]. One investigator [18] found that sex biases in drug PK strongly predicted sex-specific AEs in females and were not always explained by body weight differences. While many AEs can be linked to PK differences between females and males, surprisingly, an unidentified portion cannot, implying factors such as cultural differences, including higher AE reporting by females [19] or greater polypharmacy use among females [20, 21].

Sex differences in drug pharmacodynamics (PD) or inherent response to medications that are not attributable to PK differences have been less studied but have been observed for certain drug classes [22–24], including central nervous system (CNS) drugs such as antipsychotics, opioids, antidepressants, and ASMs [25, 26]. Evidence of differences in drug efficacy between sexes, particularly for ASMs, remains lacking [26], although such differences have been hinted at in recent publications [27–29] in patients with idiopathic generalized epilepsy (IGE), with statistically significant seizure remission favoring males treated with VPA [27–29]. Although patients with photosensitive epilepsy were included in these studies, the authors did not specifically examine VPA's impact on photosensitive male and female patients separately. Furthermore, it has been known for decades that females are more photosensitive than males, with a higher prevalence and sensitivity (producing a photoparoxysmal response (PPR) to a greater number of flash frequencies on electroencephalogram (EEG) in response to intermittent photic stimulation (IPS), i.e., an increased photosensitivity range) [28, 30, 31], it would seem that females with epilepsy plus photosensitivity should respond more fully to VPA than males. VPA is considered to be an excellent ASM, especially in generalized epilepsies, including patients with photosensitive epilepsy [32], alongside EEG improvements [33–35]. However, even in patients with generalized epilepsy and myoclonus, such as juvenile myoclonic epilepsy (JME), in which about 40% of patients are photosensitive, resistance to VPA treatment has been reported in up to 30% of cases [36]. Alternatively, VPA therapy was avoided in females owing to well-known VPA-induced teratogenic AEs in offspring [37–44].

Our objective was to determine whether or not between-sex differences exist for VPA PD using two EEG biomarkers, the PPR and derived standardized photosensitivity range (SPR). We hypothesized that an EEG PD difference may exist for VPA between females and males.

2 Methods

2.1 Study Design

We investigated whether PD differences might exist for a well-known drug, VPA, in photosensitive epilepsy. We conducted a single-center, retrospective, within-patient cohort EEG analysis in photosensitive females and males on two separate nonrandomized occasions: when they were first diagnosed with photosensitivity (ASM-naïve)

and later, after reaching steady-state VPA treatment. We evaluated each patient's response to VPA therapy by examining the change in their photosensitive hertz (Hz) range. We further transformed each patient's raw PPR range data into a SPR before and after VPA therapy and assessed potential associations with [VPA's] plasma concentrations in several patients. Finally, we explored whether age might influence the SPR response to VPA between sexes.

2.2 Rationale for the Use of EEG as a Biomarker of IPS-Induced Events

Detailed EEG studies in patients with photosensitive epilepsy have shown that the photic-induced epileptiform reaction, i.e., PPR response on EEG, can be used diagnostically [45]. Later studies demonstrated that the IPS-induced PPR on EEG could serve as an individualized biomarker for ASM treatment in clinical settings, where broader individual ranges of PPR (thresholds in Hz) correlated well with the likelihood of experiencing seizures [46–49]. It is well established that VPA reduces or suppresses PPR, as first shown after single oral doses in seven out of nine photosensitive patients [50]. This has led to the practice of monitoring paroxysmal discharges on EEG as a measure of VPA's effect on paroxysmal discharges in patients with epilepsy in clinical settings [51, 52], and specifically, monitoring the PPR on EEG in phase 2a proof-of-principle (PoP) trials for ASM development or drug comparison studies [53–56]. These changes in individually determined Hz photosensitivity range thresholds following different oral doses of VPA allow quantification of the PPR suppressive effect.

2.3 Patient Selection

We retrospectively reviewed systematically collected data using these criteria:

i. Females or males with epilepsy with either epilepsy with photosensitivity or self-induced seizures.
ii. Ages 6–55 years.
iii. Data that had been collected from 1990 to 2000, in patients with epilepsy from a tertiary care center for patients with epilepsy (and photosensitivity), Stichting Epilepsie Instellingen Nederland (SEIN Health Center), Cruquius, the Netherlands, who had undergone a minimum of two standardized EEG assessments of PPR thresholds in Hz.
iv. Only generalized PPRs were used as the EEG biomarker. Seizure or epilepsy type with classification were not selection criteria for the EEG recordings; therefore, a mixed population of inpatients and outpatients was included in the analysis.
v. Patients with a "robust" PPR range on their first EEG, i.e., those who had a calculated baseline SPR value of ≥ 3, based upon low to high Hz (Sect. 2.5), ensuring that we would be able to observe a difference in ASM therapy for each patient on their second EEG, if it existed.

No sample size was calculated a priori, believing that a review spanning 20 years would yield a sufficient sample. Since photosensitivity is most common in children and tends to increase with age, peaking around puberty [30, 31], we chose patients whose second EEG was generally performed starting no earlier than 6 weeks but no greater than 2 years after initiating VPA therapy, to minimize the unintended bias of increasing photosensitivity with age.

Informed consent was obtained from all patients or their guardians prior to EEG data collection. All our data were anonymized to ensure that no individuals could be identified at any stage of the study. The study strictly adhered to the ethical guidelines outlined in the World Medical Association's Declaration of Helsinki.

2.4 Intermittent Photic Stimulation (IPS) EEG Procedure

The EEG methodology, using the gold-standard, high-quality Grass PS33 photic stimulator (flash intensity: 1 Joule per flash), was identical for all patients and intended primarily for the clinical evaluation of ASM effectiveness. Conventional surface EEG techniques were employed following the 10–20 system, with IPS testing performed according to established standards [31, 46, 48, 57–59]. The procedure involved flashing for 5 s or less over a frequency range of 2–60 Hz to determine the lower and upper threshold sensitivity while preventing seizures. This was conducted under three eye conditions: at the initiation of eye closure, with eyes closed, and with eyes open. If generalized epileptiform PPR discharges appeared, IPS was immediately discontinued to prevent clinical seizures. The patient's individual lower and upper photosensitive thresholds were recorded to quantify their range of photosensitivity—a measure of seizure risk and the effectiveness of ASM treatment [46–49]. Although most provocative flash frequencies typically lie between 10 and 30 Hz [30, 31], some of our patients were highly photosensitive, exhibiting PPR at frequencies as low as 2 Hz and as high as 60 Hz. Among the three eye conditions, eye closure is known to be the most stable and sensitive condition for diagnosing photosensitivity [31, 48]. Therefore, our analysis focused exclusively on this

eye condition. The IPS instrumentation and methodology remained consistent from 1990 to 2000. All pre- and post-VPA treatment EEGs were interpreted solely by the primary author (D.K.N.T.) in an unblinded fashion, minimizing inter-rater variability.

2.5 Normalization of EEG IPS Threshold Data in Hertz (Hz) Range

The lower-to-upper threshold Hz data for each patient were transformed into "steps," referred to as the SPR, a value without units [31, 54, 60–63]. SPR values were calculated for each patient twice, both before and after reaching steady-state [VPA]. The minimum SPR value is 0 (no PPR observed), while the maximum, using a 14-point scale from 2 to 60 Hz, is 14. Patients who responded to only one scale point received a value of 1. The EEG response, via change in SPR, was defined as the difference between SPR after VPA treatment and SPR before therapy. It is worth noting that Binnie [54] reported a maximum SPR value of 11, by using a smaller Hz-point scale than ours, but still ranging from 2 to 60 Hz.

To interpret the EEG PPR response to add-on ASM therapy, regardless of whether the patient was treatment-naïve or had received one or more ASMs, the following response categories were established on the basis of the degree of *change* in EEG SPR, using an EEG-IPS 14-point scale from 2 Hz to 60 Hz: (i) a suboptimal response to an ASM occurs when the *change* in SPR value is only −1 or −2; a pre–post ASM therapy value of 0 can occur, indicating no change/no response in photosensitivity range at all; (ii) a clinically meaningful, *adequate* response occurs when the photosensitivity range is reduced after an ASM single dose or after chronic therapy, with a change in SPR value of at least −3 up to −13. While this response indicates that the ASM may be helpful, it *may* not be optimal; (iii) a clinically meaningful, *optimal* response occurs when the photosensitivity PPR Hz range is completely eliminated. Depending on the baseline EEG SPR value, the post-add-on ASM SPR value could range from −3 to −14; (iv) a provocative response occurs when the SPR value is ≥ +1, meaning the ASM is paradoxically increasing epileptiform EEG activity and/or clinical seizures [54, 60–63]. These EEG SPR criteria have been used in phase 2a PoP ASM development trials [53, 54, 60–63]. Our analysis of the within-patient EEG PPR and SPR changes from baseline to subsequent ASM treatment assumes that each individual's PPR response, under the same eye condition, remains stable over time. Some data support the stability of PPR in the short term, allowing for its use in phase 2a PoP ASM development trials [53, 54, 60–63].

2.6 Valproate Administration

Patients were initially ASM-naive, then were chosen by their clinician, or primary author (D.K.N.T.), to receive VPA monotherapy for their condition. VPA was chosen since it was the leading ASM for treament of photosensitivity in patients with epilepsy at that time period. Clinicians used oral enteric-coated or controlled-release-Chrono VPA tablets as therapy, administered twice daily, with some patients receiving VPA three times per day. VPA total daily mg dose was totally at the discretion of the treating clinician; dose was not based upon sex, age, or weight, and patient weight data were not collected. All patients were assumed to have achieved steady-state [VPA] concentrations after a minimum of 6 weeks of treatment. Blood samples were collected in some patients at the clinician's discretion, to measure total plasma [VPA] levels, during the end of the EEG procedure or right after the second EEG was completed, about 2–2.5 h post-VPA a.m. dose; these samples were assumed to be near or at peak concentration. While adherence to individualized VPA regimens was strongly encouraged in all patients, it was not formally monitored.

2.7 Statistical Analyses

In the descriptive analyses, relative frequencies were reported for categorical variables, while the mean, standard deviation, median, and interquartile range were reported for continuous variables.

Our first co-primary outcome is the comparison of all SPR data between female and male patients in the distribution of within-patient SPR changes from pre-VPA to steady-state VPA therapy, via Wilcoxon–Mann–Whitney *U* test. Our second co-primary outcome, which focused on percent of patients with PPR elimination on their second EEG, was analyzed using Fisher's Exact test.

We planned several secondary outcomes, including: (i) the differences in the proportions of SPR change categories by sex were evaluated using the chi-squared test or Fisher's exact test, depending on the variance between groups identified in the post-tabular analysis; (ii) differences in SPR change distribution by sex, and between VPA concentration distribution, using < 50 mg/L and > 50 mg/L as the threshold, and SPR change category by sex, were assessed using the non-parametric Wilcoxon–Mann–Whitney *U* test. This followed confirmation, through a statistical test accounting for both skewness and kurtosis, that these variables did not follow a normal distribution. The association between sex and SPR change, as well as age and SPR change, was analyzed using univariate linear regression model; (iii) the mean, standard deviation, median, and interquartile range were reported for SPR changes between sexes and plasma [VPA] levels. All our statistical analyses were based upon

the "null hypothesis" that no change in EEG-SPR values would be observed between sexes. All *p*-values reported are based on two-tailed tests at a 95% confidence interval (CI). Statistical analyses were conducted using Stata-16 software [64].

2.8 Our Choice of Terminology—Sex versus Gender

Previous studies have often used the terms "sex" and "gender" interchangeably when describing differences in PK and AEs for various drugs, including ASMs. In this study, we have chosen to consistently use the term "sex," in alignment with Kim and Nafziger [65]. "Sex" refers to variations in drug-related PK, AE, and PD responses based on biological and pathophysiological factors, while "gender" pertains to changes influenced by cultural, social, or environmental circumstances. Accordingly, we have used the terms "females" and "males" to indicate biologic sex throughout this manuscript.

3 Results

3.1 General Characteristics of the Patients Acquired

Our inclusion criteria were strict, requiring patients to be without any prior history of ASM use, which very much limited eligibility. In addition, patients had to have a robust PPR Hz range of more than 2 SPR units at first visit EEG baseline, to better enable us to see any change with VPA treatment. In addition, they had to undergo a full array of IPS testing during EEG on two occasions. Nevertheless, over a 20-year period, out of 61 patient charts reviewed as eligible, 48 patients were included, while 13 patients were excluded. Our total of 48 patients included 27 females and 21 males with a mean age of 16.5 years, standard deviation (SD) ± 7.3 years, age range 8–50 years, and all were Caucasian. The ASM-naïve status of each patient diagnosed with epilepsy (very uncommon) was confirmed by multiple sources: patient self-report, verification by the EEG technician, consultation with the treating physician, and review of hospital medical records. Patients were then retested under identical IPS conditions during regular, chronic VPA therapy. No additional ASMs were prescribed to these patients during the study period. In total, 40 patients had their follow-up/second EEG visit between 6 weeks and 6 months later, while 2 patients had their second EEG visit 1 year later, and 6 patients were seen just under 2 years later. For young females, only two patients were included in our analysis that had up to 2 years between EEGs (patients no. 6 and no. 13). For young males, only four patients (nos. 6, 12, 17, and 18) had their two visits up to 1.5–2 years apart, but the latter two males had their first EEG at age 20 years and second EEG while on VPA at age 22 years—the ages of 20 and 22 years are well beyond pubertal changes that may have affected or biased their change in PPR range and SPR. Concerning the 13 patients we excluded: 5 were treated with ASMs other than VPA, 4 could not tolerate VPA (gastrointestinal disturbances) and were switched to another ASM therapy, and 2 did not return for their second EEG until more than 2 years later, 1 contracted a disease post-initial baseline EEG, and 1 was lost to follow-up.

The age and sex distribution of the patient population is displayed in Fig. 1. The group of 27 female patients, aged 8–50 years at the time of their first IPS testing, was divided into two age categories on the basis of presumed secondary sex characteristic development and onset of puberty [66]: 8–12 years ($n = 12$, 44%) and 13–50 years ($n = 15$, 56%). This breakdown is presented in Table 1. Similarly, as shown in Table 2, the 21 male patients, aged 9–34 years, were also divided into two subgroups: 9–13 years ($n = 9$, 43%) and 14–34 years ($n = 12$, 57%) [67]. The mean and median ages at the time of the first IPS testing for the 12 pre-pubertal females were 10.7 and 10.5 years, respectively. For the nine pre-pubertal males, the mean and median ages were 10.9 and 11.0 years, respectively. In the group of 15 older females, presumed to have reached puberty, and adult females, the mean and median ages were 19.3 and 16.0 years, respectively. For the 12 older males who had reached puberty and adult males, the mean and median ages were 20.2 and 19.5 years, respectively. When considering the entire group of 27 female patients, the mean and median ages at the time of the first or baseline EEG were 15.4 and 14.0 years, respectively. For the 21 male patients at the time of the first or baseline EEG, the mean and median ages were 16.2 and 15.0 years, respectively. No statistically significant *baseline* age differences were found between the two groups, as assessed by both a two-tailed independent *t*-test (*t*-test statistic = −0.36647, $p = 0.7157$) and the non-parametric Mann–Whitney *U* test (*z*-score = 0.7586, $p = 0.447$).

3.2 General Baseline EEG-SPR Data

3.2.1 Between-Sex Difference in Baseline EEG-SPR Data

At the first EEG IPS measurement (ASM-naïve), we found that 11 out of 27 females (40.7%) had a calculated SPR greater than 10, compared with 7 out of 21 males (33.3%). This difference in baseline EEG-SPR values between sexes was not statistically significant ($p = 0.60$), indicating equivalence in prevalence in PPR on EEG at baseline in our sample.

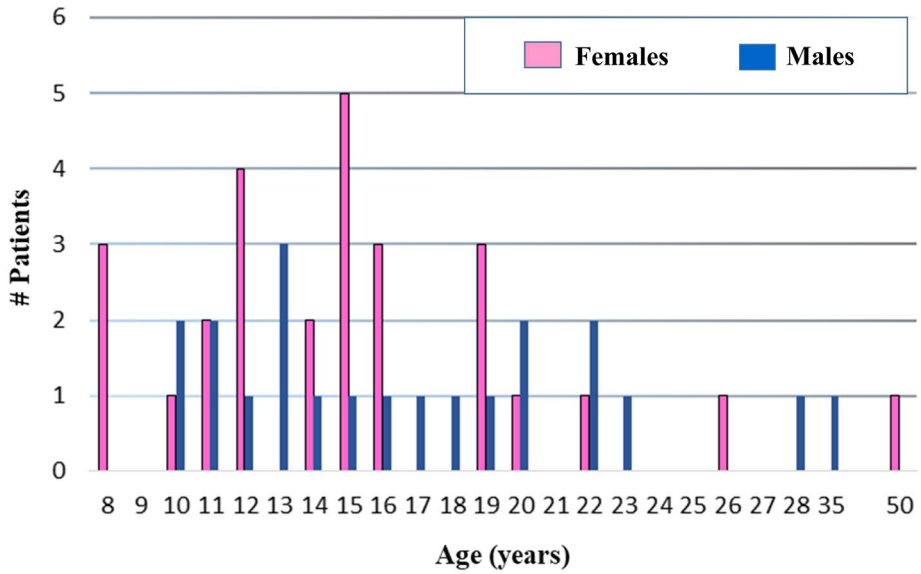

Fig. 1 Number of patients and age distribution for female and male patients with epilepsy plus photosensitivity at their first intermittent photic stimulation (IPS) procedure recorded on their electroencephalogram (EEG). The number of female patients (shown in pink) with epilepsy plus photosensitivity found on EEG in response to the IPS procedure was more frequently detected during adolescence compared with male patients (shown in blue) with epilepsy plus photosensitivity, who had a more evenly distributed age range. Yet, no statistically significant difference was observed in mean age at first IPS testing between the 27 females and 21 males (Results section)

3.2.2 Baseline EEG SPR Change by Age

Across the entire study population that included both sexes, we observed that baseline SPR increased by +0.15 points for each additional year of age (95% confidence interval (CI): +0.02 to +0.29, $p = 0.028$). However, when analyzed by sex, this age-related increase was significant only in females, with a gain of +0.19 SPR points per year (95% CI: +0.03 to +0.35, $p = 0.02$), while in males, the age-related change of +0.11 points per year was not statistically significant (95% CI: −0.09 to +0.30, $p = 0.264$).

3.3 VPA Treatment Effect on EEG-SPR

3.3.1 Overall Comparison between Sexes (First Co-Primary Outcome)

Figure 2 provides a clear depiction of the concept of the Hz photosensitivity range data collection sheet at both baseline EEG and EEG results post-VPA treatment, respectively, utilizing a 14-point scale from 2 to 60 Hz, for two of our patients: a 14-year-old female (patient no. 17, Table 1) and a 10-year-old male (patient no. 3, Table 2). The IPS was repeated 1 year later, following VPA therapy at steady state with the same daily dose of 1200 mg. The clinical history of the now 15-year-old female, who is highly photosensitive and experienced her first generalized tonic–clonic seizure (GTCS) in a nightclub, reflects the lack of efficacy of VPA. She reported: "I did not experience a reduction in my seizures or symptoms (such as pain above the eyes induced by IPS) during VPA therapy, even at a high dose." This subjective account is supported by our EEG-IPS data, which show minimal change in her photosensitivity range (6–50 Hz pre-VPA versus 8–40 Hz post-VPA) and a calculated SPR reduction of only −2 on the EEG.

The first of our co-primary outcomes, a comparison of all SPR data between female and male patients, revealed a statistically significant difference in the *distribution of within-patient SPR changes* from pre-VPA to steady-state VPA therapy. Males exhibited a significantly greater reduction in EEG-SPR pre- and post-VPA treatment compared with females. The mean reduction in females only was -3.9 ± 3.3 (median: −3, interquartile range (IQR) −1 to −7), whereas in males only, the mean reduction was -7.0 ± 2.6 (median: – 7, IQR: −5 to −9; Wilcoxon–Mann–Whitney U test, $p = 0.0018$, Fig. 3). This non-parametric test is further supported by linear regression analysis, revealing that VPA therapy resulted in a significantly greater difference in SPR values among males versus females, at a mean difference of −3.275 points (95% CI: −5.155 to −1.394, $p = 0.001$).

3.3.2 Comparison of Percent of Patients Exhibiting EEG-PPR *Elimination* between Sexes following VPA Treatment (Second Co-Primary Outcome)

Our next co-primary outcome involved PPR *elimination*. Given the clinical importance of achieving PPR elimination to maximize clinical seizure control, we analyzed the frequency of complete PPR elimination (i.e., no appearance

Table 1 Intra-patient comparison of Intermittent Photic Stimulation (IPS)-evoked photosensitivity Hertz (Hz) ranges in the eye closure condition on the electroencephalogram (EEG) before and after weeks to months of Valproic Acid (VPA) treatment in Caucasian females ($n = 27$)[a]

Patients investigated				Initial IPS/EEG results in treatment-naïve patients			Second IPS/EEG after VPA treatment in steady state					
	Patient no.	Age IPS EEG; ASM naive	Age IPS EEG after VPA	Lower EEG threshold (Hz)	Upper EEG threshold (Hz)	First calculated standardized photosensitive response (SPR)	Lower EEG threshold (Hz)	Upper EEG threshold (Hz)	Second calculated standardized photosensitive response (SPR)	Change in SPR, first to second value[b]	Total daily VPA dose (mg)	Plasma [VPA] mg/L
Females ≤ 12 years of age at first EEG	1-NR[c]	8	8	2	60	**14**	6	60	**13**	**−1**	500	124
	2	8	8	10	50	**10**	25	25	**1**	**−9**	540	107
	3	8	8	6	18	**6**	0	0	**0**	**−6**	900	–
	4	10	10	2	50	**13**	15	30	**6**	**−7**	450	–
	5	10	11	15	25	**5**	0	0	**0**	**−5**	1000	138
	6	10	12	10	50	**10**	15	30	**6**	**−4**	1250	–
	7-NR	11	11	13	30	**7**	18	30	**5**	**−2**	500	–
	8	12	12	6	40	**11**	23	30	**3**	**−8**	600	–
	9	12	12	18	40	**6**	0	0	**0**	**−6**	600	–
	10- NR	12	12	18	30	**5**	18	30	**5**	**0**	1000	80
	11	13	14	13	30	**7**	0	0	**0**	**−7**	600	–
	12	14	14	8	30	**9**	22	30	**3**	**−6**	500	–
Females ≥ 13 years of age at first EEG	13	13	15	6	50	**12**	13	15	**2**	**−10**	600	65
	14	15	15	6	50	**12**	15	20	**3**	**−9**	600	–
	15	15	15	10	25	**7**	20	22	**2**	**−5**	750	–
	16	14	15	6	40	**11**	8	15	**4**	**−7**	900	–
	17-NR	14	15	6	50	**12**	8	40	**10**	**−2**	1200	–
	18-NR	15	16	15	25	**5**	18	25	**4**	**−1**	300	–
	19-NR	15	16	8	30	**9**	10	25	**7**	**−2**	1000	118
	20	16	16	8	60	**12**	8	30	**9**	**−3**	900	89
	21-NR	18	19	13	50	**9**	15	50	**8**	**−1**	300	–
	22-NR	18	19	15	18	**2**	15	25	**5**	**+3**	900	–
	23-NR	19	19	13	30	**7**	13	30	**7**	**0**	600	–
	24-NR	19	20	10	20	**5**	10	20	**5**	**0**	600	–
	25-NR	22	22	8	50	**11**	6	50	**12**	**+1**	900	–
	26-NR	26	26	10	40	**9**	8	25	**8**	**−1**	1000	80
	27-NR	50	50	10	30	**8**	10	30	**8**	**0**	900	–
All 27 females	Average	15.4	15.9	–	–	–	–	–	–	−3.7	736.7	100.1
	± SD	8.1	8.1	–	–	–	–	–	–	3.5	257.0	25.5
	Median	14.0	15.0	–	–	–	–	–	–	−3.0	600.0	98.0

[a]Data for younger females were separated from older females on the basis of the presumed age of puberty onset, defined as an average age of < 13.0 years of age, according to Biro et al. [66]

[b]For the column titled "Change in SPR value," numerical values with a (−) sign denote a reduction in SPR value, while a (0) or "zero" indicates no change in SPR. Conversely, a (+) sign denotes an actual *increase* in SPR value, post-VPA for patient nos. 22 and 25, meaning VPA was paradoxically provocative; VPA was discontinued immediately following the IPS/EEG testing. Only 4 of 27 (14.8%) females had an elimination of PPR on their respective EEGs, with pre- and post-VPA changes in SPR values from −5 to −7. Although patient no. 13 had the largest SPR value change of −10 post-VPA among females, a PPR was still present on her second EEG post-VPA, noted by a residual SPR value = 2, indicating a merely adequate, yet non-optimal response to VPA

[c]"NR" designates a nonresponder to VPA, representing female patients who experienced an SPR decrease, post-VPA, ≤ −2 (i.e., −1 or 0), indicating a suboptimal or clinically insignificant response to VPA treatment [46–49, 54]

of PPR on EEG post-VPA, resulting in a calculated EEG-SPR value of zero for the second EEG in our patients). Complete PPR elimination was observed in 10 of 21 males (47.6%) following VPA treatment, whereas only 4 of 27 females (14.8%) achieved this outcome, representing a 3.2-fold greater degree of PPR elimination for males compared with females ($p = 0.0237$; two-tailed Fisher's exact test).

3.3.3 Between-Sex Comparison in the Percentage of Patients Meaningfully Responding to VPA across SPR Change Categories (Secondary Outcome)

One secondary outcome concerned the difference that we observed in the *percent change of patients meaningfully responding to VPA* across SPR categories between sexes. In total, 14 of 27 (51.8%) females showed a meaningful EEG-SPR change, with SPR values ranging from −3 to −10 (meaningful category). This meant that 13 of 27 (48.2%) females only displayed a nonmeaningful response to VPA therapy, defined as an SPR decrease post-VPA of only −2, −1, or 0 (Table 1). Among these, 12 patients demonstrated a modest EEG-SPR reduction ranging from −2 to 0 (suboptimal category), while 2 female patients showed an actual increase in SPR (+value), indicating a paradoxical, provocative response to VPA (SPR change values were +3 for no. 22 and +1 for no. 25, in Table 1). Table 1 also shows that 10 of 11 females older than 16 years were VPA nonresponders, having experienced a SPR change of only −2 or less, as compared with only 3 of 16 who were 16 years of age or younger.

In contrast, all 21 males (100%) demonstrated a meaningful EEG-SPR change in response to VPA, with values ranging from −3 to −13 (clinically meaningful to optimal categories; Table 2). This 1.93-fold greater percent of VPA responders with a clinically meaningful to optimal response in favor of males was statistically significant ($p < 0.0001$, two-tailed Fisher's exact test).

3.3.4 VPA Total Daily Dosage and Plasma [VPA] Concentrations (Secondary Outcome)

In the 27 females, the mean (± SD) VPA total daily dose at the second EEG was 736.7 ± 257 mg/day, with a median of 600 mg/day (Table 1). In contrast, the 21 males had a mean VPA dose of 847.6 ± 326.5 mg/day, with a median of 800 mg/day (Table 2). VPA was administered in two divided daily doses for 95.8% of the patients. This secondary outcome difference in total daily mg VPA dose between the sexes was not statistically significant (t-test statistic = 1.318, $p = 0.19$). Therefore, the greater VPA-induced reduction and/or elimination of PPR observed in males compared with females (Fig. 3) does not appear to be related to differences in VPA total daily dose, as the mean doses were not significantly different between sexes.

3.3.5 Relationship of SPR Changes to Plasma [VPA] Concentrations (Secondary Outcome)

We also evaluated available data on steady-state plasma total [VPA] concentrations (unbound or "free" [VPA] were not measured) to SPR change as the final secondary outcome measure. Most importantly, blood samples for [VPA] were typically drawn in the morning, several hours post-dose and just at the end of IPS testing, meaning these peak [VPA] concentrations were taken at the same time frame as the EEG. Peak plasma total [VPA] concentrations were available for only 13 patients (8 females and 5 males; Tables 1, 2 and 3), with an overall mean concentration in 13 patients of 87 mg/L (range 37–138 mg/L). When separated by sex, the mean (± SD) plasma [VPA] concentration in 8 females was 100.1 ± 25.5 mg/L (median 98 mg/L), while in 5 males, it was 66.0 ± 35.4 mg/L (median 51.0 mg/L; Table 3). When stratified by post-VPA EEG-SPR results, no significant difference ($p = 1.00$) was observed in the distribution of plasma [VPA] concentrations among females on the basis of EEG-SPR change categories ("Meaningful" versus "Suboptimal+Provocative"). In the suboptimal category, the mean plasma [VPA] concentration was 100.5 ± 23.8 mg/L, with a median of 99 mg/L (IQR 80–121). In patients with meaningful EEG-SPR change, median plasma [VPA] concentrations were 51.2% higher in females compared with males. For females, the mean (± SD) plasma [VPA] was 100.1 ± 25.5 mg/L (median 98 mg/L; IQR 77–122), whereas in males, it was 66.0 ± 35.4 mg/L (median 51 mg/L; IQR 38–85). Despite these higher plasma concentrations in females, the difference was not statistically significant (Table 3, Mann–Whitney U test, $p = 0.124$). Indeed, our small subsample size of 13 patients who had blood sampled for [VPA] is likely the reason for the lack of statistical significance for plasma [VPA] changes correlating with SPR changes.

4 Discussion

The overall main discovery of our retrospective study is the statistically significant between-sex differences observed for VPA's efficacy on the IPS-induced PPR on EEG, expressed through the transformed biomarker, SPR. Males show a better SPR response on EEG to VPA than females. We summarize three key findings that support this:

Table 2 Intra-patient comparison of intermittent photic stimulation (IPS)-evoked photosensitivity hertz (Hz) ranges, with calculated standardized photosensitivity response (SPR) values, in the eye closure condition on the electroencephalogram (EEG) before and after weeks to months of valproic acid (VPA) treatment in Caucasian males ($n = 21$)[a]

				Initial IPS/EEG results in treatment-naïve patients			Second IPS/EEG after VPA treatment in steady state					
	Patient no.	Age IPS EEG; ASM naive	Age IPS EEG after VPA	Lower EEG threshold (Hz)	Upper EEG threshold (Hz)	First calculated standardized photosensitive response (SPR)	Lower EEG threshold (Hz)	Upper EEG threshold (Hz)	Second calculated standardized photosensitive response (SPR)	*Change* in SPR, first to second value[b]	Total daily VPA dose, mg	Plasma [VPA] mg/L
Males ≤ 13 years of age at first EEG	1	9	10	15	30	**6**	25	25	**1**	**−5**	600	–
	2	9	10	13	40	**8**	0	0	**0**	**−8**	800	–
	3	10	11	6	50	**12**	25	25	**1**	**−11**	1200	–
	4	11	11	10	50	**10**	0	0	**0**	**−10**	500	85
	5	11	12	18	40	**6**	0	0	**0**	**−6**	800	–
	6	11	13	13	30	**7**	20	25	**3**	**−4**	800	–
	7	12	13	13	50	**9**	0	0	**0**	**−9**	1050	–
	8	12	13	10	30	**8**	13	20	**4**	**−4**	450	–
	9	13	14	2	40	**12**	20	25	**3**	**−9**	1000	119
Males ≥ 14 years of age at first EEG	10	14	15	6	40	**11**	18	20	**2**	**−9**	450	51
	11	15	16	18	40	**6**	0	0	**0**	**−6**	600	38
	12	16	18	13	30	**7**	0	0	**0**	**−7**	600	37
	13	16	17	15	40	**7**	0	0	**0**	**−7**	900	–
	14	18	19	8	40	**10**	10	10	**1**	**−9**	600	–
	15	19	20	13	20	**4**	0	0	**0**	**−4**	1800	–
	16	20	20	2	60	**14**	30	30	**1**	**−13**	900	–
	17	20	22	13	30	**7**	18	25	**4**	**−3**	900	–
	18	20	22	10	25	**7**	0	0	**0**	**−7**	900	–
	19	23	23	6	30	**10**	10	25	**7**	**−3**	1350	–
	20	28	28	13	25	**6**	0	0	**0**	**−6**	600	–
	21	34	35	13	25	**6**	23	23	**1**	**−5**	1000	–
All 21 males	Average	16.2	17.2							**−6.9**	847.6	66.0
	± SD	6.4	6.4							**2.7**	326.5	35.4
	Median	15	16							**−7.0**	800.0	51.0

[a]Data for males under 13 years of age were separated from males over 14 years of age, all Caucasian, on the basis of the presumed age of puberty onset. Puberty was defined as an average age of over 14.0 years, when 100% of Caucasian males have developed secondary sexual characteristics (Tanner Stage 2 or later), according to Herman-Giddens [67]

[b]For the column titled "Change in SPR value," numerical values with a (−) sign denote some reduction in SPR value, while a (0) or "zero" indicates no change in SPR. All male patients had a SPR value deemed to be a clinically meaningful response to VPA (i.e., SPR values from −3 to −13). In 10 of 21 males (47.6%), VPA therapy eliminated any PPR on EEG, resulting in large negative values for change in SPR. No male had a paradoxically provocative response to VPA

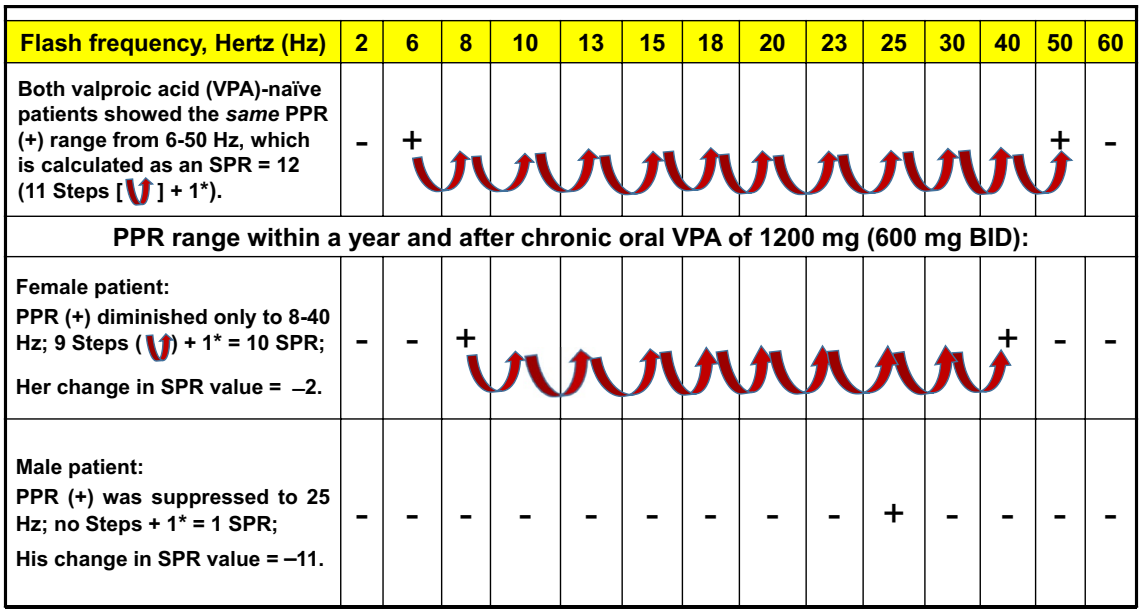

Fig. 2 Depiction of a photosensitivity range on a 14-point hertz (Hz) scale in two patients with epilepsy during baseline, while antiseizure medicine (ASM)-treatment naïve, and then at follow-up IPS-EEG procedure after chronic valproic acid (VPA) treatment. This worksheet depiction of the photosensitivity range on a 14-point Hz scale, is typically used in phase 2a proof-of-principle (PoP) ASM development trials and not uncommonly in clinical practice. The standardized photosensitivity range (SPR) value, a pharmacodynamic (PD) EEG outcome parameter calculated from the photoparoxysmal response (PPR) range, is illustrated on our worksheet determination for a 14-year-old female (no. 17 in Table 1) and a 10-year-old male (no. 3 in Table 2), both of whom were ASM-naïve. IPS testing was conducted from 2 Hz upward and 60 Hz downward until generalized epileptiform discharges were recorded. Both patients had an identical baseline EEG PPR range of 6–50 Hz (the appearance of the PPR on EEG is marked by a "+" symbol). That PPR range was then transformed into a unitless SPR value (number of interval Hz changes, or "steps," indicated by the red swooping arrow symbol ( ), then adding +1 for the final SPR value). The asterisk (*) indicates that a +1 needs to be added to the number of steps to arrive at an accurate SPR value; the maximal SPR value = 14 using this 14-point Hz range scale of 2–60 Hz. Both patients had a calculated baseline SPR value = 12 (top of illustration). Subsequently, 1 year later, after 1200 mg/day VPA treatment for both, the female's photosensitivity range reduced to 8–40 Hz (9 steps +1, for a post-VPA SPR value = 10). Thus, from baseline to post-VPA treatment, the change in the female patient's SPR value = 12 − 10 = −2. The minus sign for the value of −2 indicates that the SPR value has decreased post-VPA treatment. For the male, who exhibited a single positive generalized PPR only at 25 Hz (with no steps, but a SPR value = 1) post-VPA, the corresponding pre-post SPR values are 12 − 1 = −11. The male had a substantial, clinically meaningful, adequate response to VPA therapy, since his change in SPR value of −11 well exceeded −3, whereas the female had a suboptimal response with a SPR value of −2 (Methods, Sect. 2.5, contains more details).

i. For our first co-primary outcome, the distribution of within-patient SPR changes from pre-VPA to steady-state VPA therapy between female and male patients was statistically significantly different: mean reduction in males only was −7.0 ± 2.6 (median: −7, IQR −5 to −9), whereas in females only, the mean reduction was only −3.9 ± 3.3 (median: −3, IQR −1 to −7; p = 0.0018, Wilcoxon–Mann–Whitney U test, Fig. 3). The data above are also supported by linear regression, which showed that males had a greater difference in decrease in SPR of −3.275 points (95% CI: −5.155 to −1.394; p = 0.001).

ii. Our second co-primary outcome, PPR *elimination*, was observed in 10 out of 21 males (47.6%) following VPA treatment, whereas it was observed in only 4 out of 27 females (14.8%), a 3.2-fold difference in favor of males (p = 0.0237; two-tailed Fisher's exact test);

iii. Concerning the percentage of patients responding to VPA, a secondary outcome measure, we discovered that all 21 (100%) males had a clinically meaningful and/or optimal EEG response to VPA (change in SPR value from baseline EEG to post-VPA EEG of −3 to −13) compared with 14 of 27 (51.8%) females, a statistically significant difference in VPA response between sexes (p < 0.0001, two-tailed Fisher's exact test). This represents a 1.93-fold greater percent of VPA meaningful responders among males versus females.

These three key findings occurred despite no statistical VPA total daily milligram dose differences between sexes

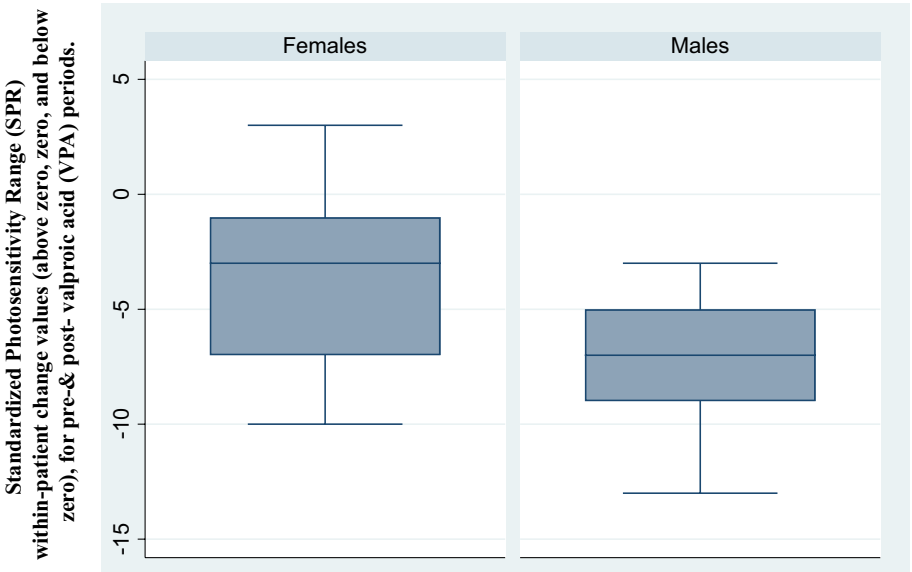

Fig. 3 Distribution of within-patient SPR differences for males and females before and after VPA therapy. This graph displays SPR value differences (box plots showing minimum, maximum, interquartile ranges (IQR), and median [as horizontal middle line]) between 27 females and 21 males before and after VPA therapy. Males exhibited a greater reduction in SPR post-VPA compared with females. A desirable clinical outcome is an SPR value decrease of −3 to −14 post-ASM treatment; an optimal response is total elimination of the PPR range post-ASM therapy. A change of SPR value, pre-to-post VPA therapy, of zero means no response to ASM treatment; any SPR change post-ASM that is more than the patient's baseline, resulting in a positive (+) value, indicates that the ASM is paradoxically provocative (Methods, Sect. 2.5, contains further details). Males exhibited a significantly greater reduction in EEG-SPR pre- and post-VPA treatment compared with females. The mean reduction in the cohort of females was −3.9 ± 3.3 (median: −3, interquartile range (IQR) −1 to −7), whereas in male group, the mean reduction was −7.0 ± 2.6 (median: −7, IQR: −5 to −9) (Wilcoxon–Mann–Whitney *U* test, $p = 0.0018$). This non-parametric test is further supported by linear regression analysis, revealing that VPA therapy resulted in a statistically significantly greater change in SPR values among males versus females post-VPA therapy, with a mean difference of −3.275 points (95% CI: −5.155 to −1.394, $p = 0.001$)

and higher plasma [VPA] concentrations in females compared with males (nonsignificant difference), in the few patients with blood samples drawn.

In most studies, photosensitivity in epilepsy, defined as the greater prevalence of PPR on EEG, occurs more often in adolescent females than in males [28, 30, 31], with females exhibiting a broader photosensitivity Hz range [46]. Thus, our findings of a greater male-to-female VPA response are surprising, given the above published statements. One might have expected females to have had the better PD response to VPA, with their broader Hz range affording a greater opportunity for VPA-induced reduction or elimination in Hz range and SPR. However, our data only found 7% more females with an SPR > 10 in the baseline EEG compared with males (albeit, a nonsignificant difference [$p = 0.60$]). The three main between-sex differences in VPA response, enumerated above, cannot be explained by age or VPA dosage, as both were statistically similar between the sexes at baseline. Females had higher average and median total plasma [VPA] concentrations than males (yet non-statistically significant at $p = 0.124$), theoretically giving females an advantage in the chance for better VPA efficacy.

To anchor our data, we relied on the EEG biomarker, the generalized PPR, with transformation to the SPR, before and after chronic VPA treatment. A clinically meaningful response using the "Photosensitivity EEG Model" is defined as a decrease in SPR value by at least 3, recorded as a negative number (−3 to −14) [46–49, 54]. The EEG-SPR serves as a robust, substantiated marker for ASM PD and/or efficacy, including VPA [31, 50–54, 60–63, 68]. Within-patient changes in Hz photosensitivity, expressed through standardized photosensitivity response (SPR), have previously been used repeatedly and successfully in phase 2a PoP ASM development trials as a surrogate marker for ASM clinical efficacy [53, 60–63].

Our findings focus on intra-patient between sex differences in PPR, converted to SPR—an EEG biomarker of PD response to ASMs, which is the first study of its kind to do so. Three other studies [27–29] have hinted that a between-sex difference in VPA response exists on the basis of changes in seizure frequency or remission. We note that our findings are in line with those from Irelli et al. [27]; they found that seizure remission in their IGE population was statistically greater ($p < 0.001$) in males (81.4%) versus females (62.3%), perhaps associated with a 50% lesser prescribing

Table 3 Focused comparison of available total plasma valproic acid [VPA] concentrations for eight females versus five males at the time of the second electroencephalographic/intermittent photic stimulation procedure (EEG/IPS), showing a 92.2% greater median plasma [VPA] concentration in female patients compared with male patients

Females assigned an identification no., as per Table 1, with a designation of (Y) as to being younger or (O) older; $n = 8$			Measured total peak (presumed) plasma [VPA] at the time of second EEG/IPS		Males assigned an identification no., as per Table 2, with a designation of (Y) as to being younger or (O) older; $n = 5$		
			Females	Males			
ID no.	1-NR[a]	Y	124	85	ID no.	4	Y
	2	Y	107	119		9	Y
	5	Y	138	51		10	O
	10-NR	Y	80	38		11	O
	11	O	65	37		12	O
	19-NR	O	118	–	–		
	20	O	89	–	–		
	26-NR	O	80	–	–		
Mean[b]:			100.1	66.0			
± SD:			25.5	35.4			
% CV:			25.4	53.7			
Median:			98.0	51.0			

[a]As in Table 1, "NR" denotes a VPA nonresponder, meaning those female patients who had an EEG-standardized photosensitive response (SPR) change value, post-VPA, of 0, or −1, or −2, indicating nonoptimal, clinically irrelevant response to VPA treatment (examples where the EEG photoparoxysmal response (PPR) range, pre- and post-ASM, has been transformed into SPR data in ASM proof-of-principle (PoP) phase 2a ASM trials include these works [46–49, 60, 61, 68]). Note that all five males meaningfully responded to VPA treatment, even for two patients whose plasma [VPA] concentrations are not generally considered to be therapeutic (< 50 mg/L)

[b]The difference between median plasma [VPA] concentrations, for eight females versus five males, although noticeably higher in females than for males, is nonsignificant, $p = 0.124$ (two-group Mann–Whitney U test, where z-statistic = 1.539 and $U = 31$)

of VPA in females versus males ($p < 0.001$). However, the prevalence of photosensitivity, via PPR on EEGs in their cohort was only listed at baseline and was statistically indistinguishable between sexes, at 29.9% for males and 28.2% for females; they also did not report on whether changes occurred within-patients' EEG PPRs over time with VPA therapy. Asadi–Pooya et al. [28], in a population of patients with epilepsy with IGE, reported a statistically significant between-sex difference in seizure remission ($p = 0.02$), with 83 (66%) males being seizure-free compared with 108 females (49%); they also found a higher proportion of males (88%) on chronic VPA therapy versus 49% of females ($p = 0.0001$). We surmise that the population of females in their study had been prescribed VPA less frequently to avoid VPA-induced teratogenic effects in offspring born to pregnant females with epilepsy [37–44]. While they did not specifically test for a cause–effect interaction, their data strongly hint that VPA appears to be more effective in males. Kilic et al. [29], in their retrospective study of patients with JME, achieved a higher seizure remission rate at 57% in 70 males with JME in the first 5 years of VPA treatment (presumably monotherapy) from 2010 to 2015, compared with 51 females (52%; $p = 0.04$) compared with the latter 5 years, 2015–2020, where levetiracetam was the predominant treatment. EEG remission was not significant in their study, however. These mentioned patient population studies [27–29] compliment our within-patient EEG study that strongly suggest a between-sexes difference in VPA PD or efficacy. We believe that a prospective, randomized trial, incorporating both EEG photosensitivity measurements plus seizure counts from multiple centers should be conducted to precisely discern the differential effect of chronic VPA treatment between sexes.

Our data on VPA total daily milligram dose and plasma total [VPA] concentrations deserves special mention. We observed biomarker EEG-SPR differences between sexes despite comparable (non-statistically significant) mean daily VPA doses between females (736.7 mg/day, Table 1) and males (847.6 mg/day, Table 2). While only 13 (27.1%) of the total 48 patients had blood samples available for VPA analysis, females had 92.2% higher median plasma [VPA] concentration compared with males (98 mg/L versus 51 mg/L, respectively; Table 3), albeit this difference was nonsignificant. Two males, with plasma [VPA] levels considered low therapeutically (37 mg/L for no. 11 and 38 mg/L for no. 12), still exhibited a VPA effect (Tables 2 and 3). The disparity between females and males in plasma [VPA] concentrations likely results from differences in body weight, as males typically weigh more than females at or post-puberty, leading to a larger absolute distribution volume and lower

total plasma [VPA] concentrations. Ibarra et al. [12] similarly found higher plasma [VPA] in females at comparable single doses, but a major flaw was not accounting for body weight differences among females and males. Yet, recent evidence suggests no significant between-sexes difference in overall plasma [VPA] or VPA pharmacokinetics (absorption, metabolism, elimination), excluding pregnancy [13]. The acute EEG photo-suppressive effect of VPA begins at a plasma [VPA] level of 53 mg/L [69], consistent with the therapeutic range of 50–100 mg/L used for seizure control in epilepsy management, underlining the heightened sensitivity to lower plasma [VPA] in males observed in our study. In addition, females have been shown to have a slightly higher plasma-free [VPA] fraction during chronic monotherapy [70], theoretically giving females an advantage in VPA efficacy. Despite not measuring free or unbound [VPA] concentrations in our study, we have no reason to suspect that the free fraction in females was lower than that in males. Any future prospective trial examining between-sex VPA efficacy differences needs to concentrate on the impact of total and free plasma [VPA] concentrations.

A growing body of literature exists on sex differences in drug PK [1–8], particularly for ASMs, including VPA [9–13]. Evidence increasingly shows that drug-induced AEs are more frequent in females across all drug classes [14–24], including ASMs [25, 26]. When it comes to ASM efficacy or PD, our data and those from a number of authors [27–29] indicate VPA response may be different between females and males, but evidence for other ASMs has not been clearly identified [26, 71–73]. A related study on the gamma-aminobutyric acid (GABA)-ergic drug diazepam found sex differences in EEG response, with 5 mg of diazepam decreasing alpha waves and increasing beta waves in both sexes and also reducing theta waves in females [74]—although the clinical significance of this is unclear. In a quantitative EEG study of ASM-naïve patients with IGE (11 females and 5 males), VPA decreased theta and delta power bands irrespective of dose or plasma [VPA] [75], but no sex differences were reported. Rowan et al. [50] documented a delayed onset of VPA's photo-suppressive effect in seven out of nine photosensitive patients with epilepsy, with maximal suppression occurring 3 h post-VPA dose, most likely at peak concentrations. Five of these patients, on chronic VPA and other ASMs, did not show a reduction in photosensitivity. In one 53-year-old female patient, the initial decrease in the EEG photosensitivity range, induced by oral VPA, was notable. Over 5 days, her range decreased from 20 to 10 Hz (a reduction in the upper-lower Hz threshold). However, this improvement was not sustained over time, as her photosensitivity range slowly reverted to its original value of 20 Hz. We did not conduct serial testing of VPA's photo-responsiveness in our patients, so it is possible that the female patients in our study initially responded to VPA but later became refractory, as observed in Rowan's patient. Our study extends Rowan's findings by detailing a significant sex difference in VPA's suppressive effect on the PPR in a larger cohort of patients. It also reminds us that, perhaps, VPA resistance might occur over time in some patients, such that a proper future study design should incorporate more frequent clinical and EEG assessments to discern between-sex differences.

We speculate there may be several reasons to explain the VPA refractoriness that we observed in nearly half of the females in our study with photosensitive epilepsy. Whether females exhibit a lower response to VPA or males demonstrate heightened sensitivity to the drug likely stems from inherent physiological factors unrelated to VPA PK.

First, one possible factor could be the influence of female hormones and menstrual cycles, especially considering that 10 of 11 females at ≥ 16 years of age were VPA nonresponders, whereas only 3 females < 16 years displayed this nonresponder effect (Table 1). This observation plus the result of our first co-primary outcome, the distribution of SPR change from pre-VPA to steady-state VPA therapy between female and male patients being remarkably different ($p = 0.0018$, Wilcoxon–Mann–Whitney U test, Fig. 3) and a secondary outcome—the percentage of males with a VPA clinically meaningful to optimal response being 1.93-fold greater than females, at 100:51.8%, respectively ($p < 0.0001$), strongly suggests that VPA nonresponse is heavily influenced by estrogen/progesterone hormones. The role of such female hormones in epilepsy is both complex and contradictory. Estrogens are known to have both proconvulsant and anticonvulsant properties [76–78]. Moreover, birth control pills (BCPs) containing estrogen and progesterone have been shown to statistically decrease plasma [VPA] by 23.4% in females with epilepsy [79]. Yet, in our study, female patients exhibited higher plasma [VPA] concentrations than males. The influence of BCPs on VPA responsiveness in females certainly needs further study.

Secondly, it is possible that VPA metabolites contributed differently to drug efficacy between sexes, potentially influencing our findings. Clinical observations by Rowan et al. [50] of delayed onset of VPA's effect on PPR suppression (occurring hours after peak plasma [VPA] levels) and its persistence for up to 5 days suggest that VPA metabolites may play a role in its efficacy. Loscher et al. [80] examined five VPA metabolites (2-ene-VPA, 3-OH-VPA, 4-OH-VPA, 5-OH-VPA, and 3-keto-VPA) in 29 patients with epilepsy on chronic VPA therapy. With a mean plasma [VPA] of 105 mg/L, the percentage of these metabolites ranged from ~6% for 2-ene-VPA to ~0.7% for 4-OH-VPA, with others falling in between. When studied in mice, these metabolites were found to elevate seizure thresholds significantly, though they were 50–90% less potent than VPA itself. The researchers concluded that VPA accounted for ~80% of the antiepileptic effect during chronic therapy in humans, suggesting

that VPA metabolites do not substantially contribute to its efficacy at the plasma concentrations observed in humans, regardless of sex differentiation [81].

Thirdly, an early review [82] of drug efflux transporters, including multidrug-resistant protein, p-glycoprotein (Pgp), and organic anion-transporting proteins, explored their role in drug resistance in central nervous system (CNS) disorders, including epilepsy. It was clearly shown that VPA is not a substrate for Pgp [83], and while no between-sex differences in brain drug transporters have been reported, this premise does not seem likely to explain our results.

Fourthly, VPA is known to increase [GABA] levels in the cerebrospinal fluid and brain, enhance GABA synthesis, decrease its degradation, and promote its release, which inhibits excitatory neurotransmission and reduces seizure activity [84–87]. Of the four major reviews on this topic, only one study [86] mentioned potential sex differences in GABA receptor distribution or function. An entire book dedicated to sex differentiation of the CNS, including a chapter on epilepsy [73], discusses the influence of menstruation, neuroendocrine hormones, and biological differences in neuronal networks but provides no evidence of differences in GABA receptor number, distribution, or function between males and females. Fifthly, a recent novel investigation [88] has found that genetic overexpression of the scaffolding protein, Homer 1b/c, in the post-synaptic density of hippocampal neurons, induces VPA resistance in rats via a number of mechanisms, including increasing production of reactive oxygen species, calcium content or facilitating lactate dehydrogenase release. Yet, the lack of data for both sexes of rats limits our understanding of the potential for an abundance of Homer 1b/c to disrupt VPA efficacy; this needs further exploration.

4.1 Limitations

We recognize that our study has a number of limitations. It was an unblinded, nonrandomized, and retrospective work. While our sample size may be considered small since it only included a single-center, it included a relatively large cohort of ASM-naïve patients with epilepsy, which are difficult to identify and systematically follow using standardized photic stimulation in contemporary neurology practice. Although we precisely determined individual, within-patient changes in photosensitivity Hz range and SPR before and after VPA treatment, we did not correlate these biomarker PPR changes with actual clinical seizure outcomes for this cohort, such as seizure frequency being recorded in seizure diaries or through periodic video EEG monitoring. Yet, we note that this correlation has been established and published before [46–49]. We recognize that our findings, being from a single center with a small sample size and having had only one race, Caucasian participants, limits the generalizability of our data; yet, we found strong statistical significance for both our co-primary and some secondary outcomes. Indeed, we welcome prospective data from multiple sites to confirm our observations. We also did not record patients' body weights, which would have provided more accurate mg/kg VPA dosing information by sex. Blood samples for plasma [VPA] concentrations were collected in only 13 of 48 patients, and the very exact timing of these collections after IPS testing (when plasma [VPA] would be maximized) may have led to variability in [VPA] concentrations between patients. Furthermore, we did not measure the free fraction of plasma [VPA], nor did we track patient adherence to individualized VPA regimens. The response to VPA treatment was not measured at a consistent time point after initiation of therapy, other than that we allowed a minimum of 6 weeks of VPA therapy to attain PK and PD steady-state conditions. Although all patients were on VPA monotherapy, we did not systematically monitor the concomitant use of other non-seizure medications or nutraceuticals, including the use of BCPs in females. Any potential drug–drug or drug-nutrient interactions may have unknowingly hindered VPA efficacy in females or perhaps enhanced the response in males. We did not document the timing of menstrual cycles relative to the first or second EEG IPS testing, nor did we track between-sex differences in AEs. However, patients received appropriate clinical management, with VPA doses adjusted when nuisance AEs occurred. Lastly, our cohort involved patients with epilepsy plus photosensitivity, and some readers might argue that our results only pertain to this specific population alone—yet, it begs the question as to whether or not sex differences might be seen in the general population of VPA-treated patients with epilepsy. We remind readers that these same EEG SPR criteria used in our cohort herein to evaluate VPA effect have also been used in many other phase 2a PoP ASM development trials [53–56, 60–63], and a number of those ASMs have ultimately shown broad clinical efficacy and have been US Food and Drug Administration (FDA)-approved for the treatment of patients with epilepsy *with* or *without* photosensitivity.

5 Conclusions

We observed significant between-sex differences in response to VPA via the pharmacodynamic biomarker, the EEG PPR (with transformation to SPR units): males showed a greater reduction in SPR, a higher rate of PPR elimination than females, despite comparable VPA total daily milligram doses, attendant with almost twice higher (but nonsignficant) plasma [VPA] concentrations in females. All 21 males

had a clinically meaningful and/or optimal EEG-SPR VPA response compared with 51.8% of females. These findings suggest that inherent biological or physiologic sex differences may influence VPA efficacy by an unknown mechanism; this certainly requires further study. Furthermore, our data emphasizes the importance of considering sex in personalized ASM treatment planning for patients with epilepsy plus photosensitivty.

Acknowledgements We are especially grateful to J. Stroink, MD for referring many patients for clinical evaluation on the basis of their history of visually evoked seizures.

Declarations

Ethics Approval and Consent to Participate Conducting IPS in any patient with epilepsy plus a claim of clinical photosensitivity has been the standard of medical care since the 1950s and still is today [References A, B]. IPS testing is considered a safe tool for diagnosis and evaluation of effect of antiseizure medication when applied correctly [Reference C]. All patients that have been or presently are referred to a tertiary care hospital in the Netherlands have to give written, informed consent from the patient or guardian prior to the diagnostic tests. We retrospectively mined de-identified patient data from 1990 to 2000 for analysis. These type of clinically gathered data were considered exempt from IRB review. This study was conducted in strict accordance with the ethical principles outlined in the World Medical Association's Declaration of Helsinki. A. Tatum WO, Rubboli G, Kaplan PW, Mirsatari SM, Radhakrishnan K, Gloss D, Caboclo LO, Drislane FW, Koutroumanidis M, Schomer DL, Kasteleijn-Nolst Trenité D, Cook M, Beniczky S. Clinical utility of EEG in diagnosing and monitoring epilepsy in adults. Clin Neurophysiol 2018 May; 129(5):1056-1082. B. Kasteleijn-Nolst Trenité D, Rubboli G, Hirsch E, Martins da Silva A, Seri S, Wilkins A, Parra J, Covanis A, Elia M, Capovilla G, Stephani U, Harding G. Methodology of photic stimulation revisited: updated European algorithm for visual stimulation in the EEG laboratory. Epilepsia 2012 Jan; 53(1):16-24. https://www.ilae.org/files/ilaeGuideline/PhoticStimulation-2012-.1528-1167.2011.03319.pdf. C. Whitehead K, Sherratt M, Kandler R, Lawrence S, Pang C. Photic stimulation during electroencephalography: efficacy and safety in an unselected cohort of patients referred to UK neurophysiology departments. Seizure 2016 Jan; 34:29-34.

Consent for Publication Not applicable. Also note: Large Language Models (LLMs; e.g., ChatGPT), were not utilized in the initial, subsequent drafts, or final version of our manuscript.

Code Availability Not applicable.

Author Contributions Dorothee Kasteleijn-Nolst Trenité (D.K.N.T.)—conceptualization, data curation, writing—original draft, and writing—review and editing; Ronald C. Reed (R.C.R.)—conceptualization, data curation, data analysis, statistical analysis, writing—original draft, and writing—review and editing; Alessandro Ferretti (A.F.)—data analysis, statistical analysis, visualization, writing—original draft, and writing—review and editing; Anteo di Napoli (A.D.N.)—data analysis, statistical analysis, visualization, writing—original draft, and writing—review and editing; Pasquale Parisi (P.P.)—supervision and writing—review and editing.

Funding The authors declare that this study received no financial support from internal or external resources.

Competing Interests All authors declare no financial, nor non-financial, conflicts of interest.

Availability of Data and Materials Upon justifiable request, data that supports the findings of this study may be obtained by contacting either the corresponding author, Ronald C. Reed, or the primary author, Dorothee' G.A. Kasteleijn-Nolst Trenité.

Abbreviations Appearing in the Abstract, Key Points, Text, Tables, Figures and Figure Legends, Listed in Alphabetical Order *AEs* adverse effects, *ASMs* antiseizure medicines, *BCPs* birth control pills, *CNS* central nervous system, *CI* confidence interval, *EEG* electroencephalogram or electroencephalographic, *GABA* gaba-amino-butyric acid, *Hz* hertz, *IGE* idiopathic generalized epilepsy, *IPS* intermittent photic stimulation, *IQR* interquartile range, *JME* juvenile myoclonic epilepsy, *PD* pharmacodynamics, *PK* pharmacokinetics, *PPR* photoparoxysmal response, *PoP* proof-of-principle, *SPR* standardized photosensitivity range, *SEIN Health Center, NL* Stichting Epilepsie Instellingen Nederland, STD standard deviation, *VPA* valproic acid

References

1. Anderson GD. Sex differences in drug metabolism: cytochrome P-450 and uridine diphosphate glucuronosyltransferase. J Gend Specif Med. 2002;5(1):25–33.
2. Schwartz JB. The influence of sex on pharmacokinetics. Clin Pharmacokinet. 2003;42(2):107–21. https://doi.org/10.2165/00003088-200342020-00001.
3. Schwartz JB. The current state of knowledge on age, sex, and their interactions on clinical pharmacology. Clin Pharmacol Ther. 2007;82(1):87–96. https://doi.org/10.1038/sj.clpt.6100226.
4. Soldin OP, Mattison DR. Sex differences in pharmacokinetics and pharmacodynamics. Clin Pharmacokinet. 2009;48(3):143–57. https://doi.org/10.2165/00003088-200948030-00001.
5. Soldin OP, Chung SH, Mattison DR. Sex differences in drug disposition. J Biomed Biotechnol. 2011;2011: 187103. https://doi.org/10.1155/2011/187103.
6. Franconi F, Campesi I. Pharmacogenomics, pharmacokinetics and pharmacodynamics: interaction with biological differences between men and women. Br J Pharmacol. 2014;171(3):580–94. https://doi.org/10.1111/bph.12362.
7. Chen M-L, Lee SC, Ng MJ, Schuirmann DJ, Lesko LJ, Williams RL. Pharmacokinetic analysis of bioequivalence trials: implications for sex-related issues in clinical pharmacology and biopharmaceutics. Clin Pharmacol Ther. 2000;68(5):510–21. https://doi.org/10.1067/mcp.2000.111184.
8. Harris RZ, Benet LZ, Schwartz JB. Gender effects in pharmacokinetics and pharmaco-dynamics. Drugs. 1995;50(2):222–39. https://doi.org/10.2165/00003495-199550020-00003.

9. Marino SE, Birnbaum AK, Leppik IE, Conway JM, Musib LC, Brundage RC, et al. Steady-state carbamazepine pharmacokinetics following oral and stable-labeled intravenous administration in epilepsy patients: effects of race and sex. Clin Pharmacol Ther. 2012;91(3):483–8. https://doi.org/10.1038/clpt.2011.251.
10. Johannessen-Landmark C, Beiske G, Baftiu A, Burns ML, Johannessen SI. Experience from therapeutic drug monitoring and gender aspects of gabapentin and pregabalin in clinical practice. Seizure. 2015;28:88–91. https://doi.org/10.1016/j.seizure.2015.02.017.
11. Yukawa E, To H, Ohdo S, Higuchi S, Aoyama T. Population-based investigation of valproic acid relative clearance using nonlinear mixed effects modeling: influence of drug-drug interaction and patient characteristics. J Clin Pharmacol. 1997;37(12):1160–7. https://doi.org/10.1002/j.1552-4604.1997.tb04301.x.
12. Ibarra M, Vázquez M, Fagiolino P, Derendorf H. Sex related differences on valproic acid pharmacokinetics after oral single dose. J Pharmacokinet Pharmacodyn. 2013;40(4):479–86. https://doi.org/10.1007/s10928-013-9323-3.
13. Gu W, Reed RC, Dutta S, Hosmane BS and Haywood A. Do pharmacokinetic sex differences for valproate exist between men and women?: A retrospective, pooled analysis from five prospective studies. (abstract/poster) American Society of Health-System Pharmacy (ASHP) 2022 Midyear Clinical Meeting, Las Vegas, NV, 4–8 December 2022; (submitted, with Dutta S as 1st author, June 2025).
14. Yu Y, Chen J, Li D, Wang L, Wang W, Liu H. Systematic analysis of adverse event reports for sex differences in adverse drug events. Scientific Rep. 2016;6:24955. https://doi.org/10.1038/srep24955.
15. Tharpe N. Adverse drug reactions in women's health care. J Midwifery Womens Health. 2011;56(3):205–13. https://doi.org/10.1111/j.1542-2011.2010.00050.x.
16. Martin RM, Biswas PN, Freemantle SN, Pearce GL, Mann RD. Age and sex distribution of suspected adverse drug reactions to newly marketed drugs in general practice in England: analysis of 48 cohort studies. Br J Clin Pharmacol. 1998;46(5):505–11. https://doi.org/10.1046/j.1365-2125.1998.00817.x.
17. Brabete AC, Greaves L, Maximos M, Huber E, Li A, Le M-L. A sex- and gender-based analysis of adverse drug reactions: a scoping review of pharmacovigilance databases. Pharmaceuticals (Basel). 2022;15(3):298. https://doi.org/10.3390/ph15030298.
18. Zucker I, Prendergast BJ. Sex differences in pharmacokinetics predict adverse drug reactions in women. Biol Sex Differ. 2020;11(1):32. https://doi.org/10.1186/s13293-020-00308-5.
19. Kando JC, Yonkers KA, Cole JO. Gender as a risk factor for adverse events to medications. Drugs. 1995;50(1):1–6. https://doi.org/10.2165/00003495-199550010-00001.
20. Orlando V, Mucherino S, Guarino G, Guerriero F, Trama U, Menditto E. Gender differences in medication use: a drug utilization study based on real world data. Int J Environ Res Public Health. 2020;17(11):3926. https://doi.org/10.3390/ijerph17113926.
21. Martin CB, Hales CM, Gu Q, and Ogden CL. Prescription drug use in the United States, 2015–2016. National Center for Health Statistics (NCHS) Data Brief, No, 334, May 2019 [Fig. 1]. https://www.cdc.gov/nchs/data/databriefs/db334-h.pdf
22. Thürmann PA, Hompesch BC. Influence of gender on the pharmacokinetics and pharmaco-dynamics of drugs. Int J Clin Pharmacol Ther. 1998;36(11):586–90.
23. Beierle I, Meibohm B, Derendorf H. Gender differences in pharmacokinetics and pharmacodynamics. Int J Clin Pharmacol Ther. 1999;37(11):529–47.
24. Gandhi M, Aweeka F, Greenblatt RM, Blaschke TF. Sex differences in pharmacokinetics and pharmacodynamics. Annu Rev Pharmacol Toxicol. 2004;44:499–523. https://doi.org/10.1146/annurev.pharmtox.44.101802.121453.
25. Seeman MV. The pharmacodynamics of antipsychotic drugs in women and men. Front Psychiatry. 2021;12: 650904. https://doi.org/10.3389/fpsyt.2021.650904.
26. Romanescu M, Buda V, Adelina Lombrea A, Andor M, Ionut Ledeti I, Suciu M, Danciu C, Dehelean CA, Dehelean L. Sex-related differences in pharmacological response to CNS drugs: a narrative review. J Pers Med. 2022;12(6):907. https://doi.org/10.3390/jpm12060907.
27. Irelli CE, Cocchi E, Morano A, Casciato S, Fanella M, Albini M, et al. Valproate impact and sex-dependent seizure remission in patients with idiopathic generalized epilepsy. J Neurol Sci. 2020;415: 116940. https://doi.org/10.1016/j.jns.2020.116940.
28. Asadi-Pooya AA, Homayoun M. Sex differences in characteristics of idiopathic generalized epilepsies. Neurol Sci. 2021;42(6):2421–4. https://doi.org/10.1007/s10072-020-04834-3.
29. Kılıç B, Serdaroğlu E, Polat BG, İnce T, Esenülkü G, Topçu Y, et al. Trends in the choice of antiseizure medications in juvenile myoclonic epilepsy: a retrospective multi-center study from Turkey between 2010 and 2020. Seizure. 2022;99:48–53. https://doi.org/10.1016/j.seizure.2022.05.005.
30. Topalkara K, Alarcón G, Binnie CD. Effects of flash frequency and repetition of intermittent photic stimulation on photoparoxysmal responses. Seizure. 1998;7(3):249–55. https://doi.org/10.1016/s1059-1311(98)80044-7.
31. Timmings P and Piccioli M. What can we learn from a photosensitive patient? (Chapter 13). In 'The importance of photosensitivity for epilepsy', Springer-Nature, Switzerland AG. 2021. https://doi.org/10.1007/978-3-319-05080-5.
32. Wilder BJ, Ramsay RE, Murphy JV, Karas BJ, Marquardt K, Hammond EJ. Comparison of valproic acid and phenytoin in newly diagnosed tonic-clonic seizures. Neurology. 1983;33(11):1474–6. https://doi.org/10.1212/wnl.33.11.1474.
33. Scollo-Lavizzari G, Corbat F. A clinical note on a new antiepileptic 'Depakine.' Eur Neurol. 1970;4(5):312–7. https://doi.org/10.1159/000114032.
34. Maheshwari MC, Jeavons PM. Proceedings: the effect of sodium valproate (Epilim) on the EEG. Electroencephalogr Clin Neurophysiol. 1975;39(4):429.
35. Covanis A, Gupta AK, Jeavons PM. Sodium valproate: monotherapy and polytherapy. Epilepsia. 1982;23(6):693–720. https://doi.org/10.1111/j.1528-1157.1982.tb05085.x.
36. Fernando-Dongas MC, Radtke RA, VanLandingham KE, Husain AM. Characteristics of valproic acid resistant juvenile myoclonic epilepsy. Seizure. 2000;9(6):385–8. https://doi.org/10.1053/seiz.2000.0432.
37. Arpino C, Brescianini S, Robert E, Castilla EE, Cocchi G, Cornel MC, et al. Teratogenic effects of antiepileptic drugs: use of an international database on malformations and drug exposure (MADRE). Epilepsia. 2000;41(11):1436–43. https://doi.org/10.1111/j.1528-1157.2000.tb00119.x.
38. Meador KJ, Baker GA, Browning N, Clayton-Smith J, Combs-Cantrell DT, Cohen M, NEAD Study Group, et al. Cognitive function at 3 years of age after fetal exposure to antiepileptic drugs. N Engl J Med. 2009;360(16):1597–605. https://doi.org/10.1056/NEJMoa0803531.
39. Harden CL, Meador KJ, Pennell PB, Hauser WA, Gronseth GS, French JA, American Academy of Neurology & American Epilepsy Society, et al. Practice parameter update: management issues for women with epilepsy—focus on pregnancy (an evidence-based review): teratogenesis and perinatal outcomes: report of the Quality Standards Subcommittee and Therapeutics and Technology Assessment Sub-Committee of the American Academy of Neurology and American Epilepsy Society. Neurology. 2009;732:133–41. https://doi.org/10.1212/WNL.0b013e3181a6b312.

40. Tomson T, Battino D. Teratogenic effects of antiepileptic drugs. Lancet Neurol. 2012;11(9):803–13. https://doi.org/10.1016/S1474-4422(12)70103-5.
41. Meador KJ, Baker GA, Browning N, Cohen MJ, Bromley RL, Clayton-Smith J, et al. Fetal antiepileptic drug exposure and cognitive outcomes at age 6 years (NEAD study): a prospective observational study. Lancet Neurol. 2013;12(3):244–52. https://doi.org/10.1016/S1474-4422(12)70323-X.
42. Tomson T, Marson A, Boon P, Canevini MP, Covanis A, Gaily E, et al. Valproate in the treatment of epilepsy in girls and women of childbearing potential. Epilepsia. 2015;56(7):1006–19. https://doi.org/10.1111/epi.13021.
43. Meador KJ, Loring DW. Developmental effects of antiepileptic drugs and the need for improved regulations. Neurology. 2016;86(3):297–306. https://doi.org/10.1212/WNL.0000000000002119.
44. Tomson T, Battino D, Bonizzoni E, Craig J, Lindhout D, Perucca E, the EURAP Study Group, et al. Comparative risk of major congenital malformations with eight different antiepileptic drugs: a prospective cohort study of the EURAP registry. Lancet Neurol. 2018;17(6):530–8. https://doi.org/10.1016/S1474-4422(18)30107-8.
45. Bickford RG, Daly D, Keith HM. Convulsive effects of light stimulation in children. Am J Dis Child. 1953;86:170–83. https://doi.org/10.1001/archpedi.1953.02050080179005.
46. Kasteleijn-Nolst Trenite DG. Photosensitivity in epilepsy. Electrophysiological and clinical correlates. Acta Neurol Scand (Suppl). 1989;125:3–149.
47. Harding GF, Herrick CE, Jeavons PM. A controlled study of the effect of sodium valproate on photosensitive epilepsy and its prognosis. Epilepsia. 1978;19(6):555–65. https://doi.org/10.1111/j.1528-1157.1978.tb05036.x.
48. Trenité DG. Photosensitivity, visually sensitive seizures and epilepsies. Epilepsy Res. 2006;70(Suppl 1):S269–79. https://doi.org/10.1016/j.eplepsyres.2006.02.012.
49. Capovilla G and Gambardella A. Correlation EEG and Clinic (Chapter 6). In 'The importance of photosensitivity for epilepsy', Springer-Nature, Switzerland AG, January 2021. https://doi.org/10.1007/978-3-319-05080-5 (**Print ISBN978-3-319-05079-9, Online ISBN978-3-319-05080-5**)
50. Rowan AJ, Binnie CD, Warfield CA, Meinardi H, Meijer JW. The delayed effect of sodium valproate on the photoconvulsive response in man. Epilepsia. 1979;20(1):61–8. https://doi.org/10.1111/j.1528-1157.1979.tb04776.x.
51. Rowan AJ, Binnie CD, de Beer-Pawlikowski NKB, Goedhart DM, Gutter T, van der Geest P, et al. Sodium valproate: serial monitoring of EEG and serum levels. Neurology. 1979;29:1450–9. https://doi.org/10.1212/wnl.29.11.1450.
52. Rowan AJ, Binnie CD, de Beer-Pawlikowski NKB, Goedhart DM, Gutter T, van der Geest P et al. Serial twenty-four-hour serum level monitoring in the study of sodium valproate. In 'The place of sodium valproate in the treatment of epilepsy.' Editors: Parsonage MJ and Caldwell ADS, Royal Society of Medicine International Congress and Symposium Ser., No. 30/Academic Press, Inc., London, 1980. (**ISBN 10: 0127932488 & ISBN 13: 9780127932484**).
53. Binnie C. Proof of principle trials: EEG surrogate endpoints. Epilepsy Res. 2001;45(1–3):7–11. https://doi.org/10.1016/s0920-1211(01)00204-2.
54. Binnie CD, Kasteleijn-Nolst Trenité DG, De Korte R. Photosensitivity as a model for acute antiepileptic drug studies. Electroencephalogr Clin Neurophysiol. 1986;63(1):35–41. https://doi.org/10.1016/0013-4694(86)90060-x.
55. Yuen ES, Sims JR. How predictive are photosensitive epilepsy models as proof of principle trials for epilepsy? Seizure. 2014;23(6):490–3. https://doi.org/10.1016/j.seizure.2014.03.011.
56. Reed RC, Rosenfeld WE, Lippmann SM, Abou-Khalil B, Kasteleijn-Nolst Trenite' D. Creative use of the conventional 'human photosensitivity model in epilepsy' (Chapter 24). In 'The importance of photosensitivity for epilepsy', Springer-Nature, Switzerland AG, 2021. https://doi.org/10.1007/978-3-319-05080-5. (**Print ISBN978-3-319-05079-9, Online ISBN978-3-319-05080-5**).
57. Kasteleijn-Nolst Trenité DG, Binnie CD, Harding GF, Wilkins A. Photic stimulation: standardization of screening methods. Epilepsia. 1999;40(Suppl 4):75–9. https://doi.org/10.1111/j.1528-1157.1999.tb00911.x.
58. Fisher RS, Acharya JN, Baumer FM, French JA, Parisi P, Solodar JH, et al. Visually sensitive seizures: an updated review by the Epilepsy Foundation. Epilepsia. 2022;63(4):739–68. https://doi.org/10.1111/epi.17175.
59. Kasteleijn-Nolst Trenité D, Rubboli G, Hirsch E, Martins da Silva A, Seri S, Wilkins A, et al. Methodology of photic stimulation revisited: updated European algorithm for visual stimulation in the EEG laboratory. Epilepsia. 2012;53(1):16–24. https://doi.org/10.1111/j.1528-1167.2011.03319.x.
60. Kasteleijn-Nolst Trenite D, Genton P, Parain D, Masnou P, Steinhoff B, Jacobs T, et al. Evaluation of brivaracetam, a novel SV2A ligand, in the photosensitivity model. Neurology. 2007;69(10):1027–34. https://doi.org/10.1212/01.wnl.0000271385.85302.55.
61. Kasteleijn-Nolst Trenité D, Parain D, Genton P, Masnou P, Schwartz J-C, Hirsch E. Efficacy of the histamine 3 receptor (H3R) antagonist pitolisant (formerly known as tiprolisant; BF2.649) in epilepsy: dose-dependent effects in the human photosensitivity model. Epilepsy Behav. 2013;28(1):66–70. https://doi.org/10.1016/j.yebeh.2013.03.018.
62. Kasteleijn-Nolst Trenité D, Brandt C, Mayer T, Rosenow F, Schmidt B, Steinhoff BJ, et al. Dose-dependent suppression of human photoparoxysmal response with the competitive AMPA/kainate receptor antagonist BGG492: clear PK/PD relationship. Epilepsia. 2015;56(6):924–32. https://doi.org/10.1111/epi.13008.
63. Kasteleijn-Nolst Trenite DGA, DiVentura BD, Pollard JR, Krauss GL, Mizne S, French JA. Suppression of the photoparoxysmal response in photosensitive epilepsy with cenobamate (YKP3089). Neurology. 2019;93(6):e559–67. https://doi.org/10.1212/WNL.0000000000007894.
64. Stata Corp. 2019. Stata Statistical Software: release 16. College Station, TX: Stata Corp LLC).
65. Kim JS, Nafziger AN. Is it sex or is it gender? Clin Pharmacol Ther. 2000;68(1):1–3. https://doi.org/10.1067/mcp.2000.108356.
66. Biro FM, Greenspan LC, Galvez MP. Puberty in girls of the 21st century. J Pediatr Adolesc Gynecol. 2012;25(5):289–94. https://doi.org/10.1016/j.jpag.2012.05.009.
67. Herman-Giddens ME, Wang L, Koch G. Secondary sexual characteristics in boys: estimates from the national health and nutrition examination survey III, 1988–1994. Arch Pediatr Adolesc Med. 2001;155(9):1022–8. https://doi.org/10.1001/archpedi.155.9.1022.
68. Kasteleijn-Nolst Trenité DGA, French JA, Hirsch E, Macher J-P, Meyer B-U, Grosse PA, et al. Evaluation of carisbamate, a novel antiepileptic drug, in photosensitive patients: an exploratory, placebo-controlled study. Epilepsy Res. 2007;74(2–3):193–200. https://doi.org/10.1016/j.eplepsyres.2007.03.012.
69. Kasteleijn-Nolst Trenite D, Reed RC, Rosenfeld WE, Hosmane B and Bassel Abou-Khalil B. Preliminary evidence for a minimum effective total concentration of valproic acid in photosensitive epilepsy patients-a placebo controlled IV study. 28th International Epilepsy Congress (IEC), Budapest, Hungary, 28 June to 2 July 2009; Epilepsia 2009; 50 (Suppl 10): 105 (Poster no. 483).
70. Nasreddine W, Dirani M, Atweh S, Makki A, Beydoun A. Determinants of free serum valproate concentration: a prospective

study in patients on divalproex sodium monotherapy. Seizure. 2018;59:24–7. https://doi.org/10.1016/j.seizure.2018.04.012.
71. Perucca E. Pharmacological and therapeutic properties of valproate: a summary after 35 years of clinical experience. CNS Drugs. 2002;16(10):695–714. https://doi.org/10.2165/00023210-200216100-00004.
72. Perucca E, Battino D, Tomson T. Gender issues in antiepileptic drug treatment. Neurobiol Dis. 2014;72(Pt B):217–23. https://doi.org/10.1016/j.nbd.2014.05.011.
73. Sohrabji F. Chap. 12: Sex difference in the central nervous system, in 'Sex differences in neurologic disease,' Shansky RM, Editor, Elsevier, 2016. ISBN: 978-0-12-802114-9.
74. Romano-Torres M, Borja-Lascurain E, Chao-Rebolledo C, del-Río-Portilla Y, Corsi-Cabrera M. Effect of diazepam on EEG power and coherent activity: sex differences. Psychoneuroendocrinology. 2002;27(7):821–33. https://doi.org/10.1016/s0306-4530(01)00082-8.
75. Clemens B. Valproate decreases EEG synchronization in a use-dependent manner in idiopathic generalized epilepsy. Seizure. 2008;17(3):224–33. https://doi.org/10.1016/j.seizure.2007.07.005.
76. Veliskova J. The role of estrogens in seizures and epilepsy: the bad guys or the good guys? Neuroscience. 2006;138(3):837–44. https://doi.org/10.1016/j.neuroscience.2005.07.005.
77. Veliskova J. Estrogens and epilepsy: why are we so excited? Neuroscientist. 2007;13(1):77–88. https://doi.org/10.1177/1073858406295827.
78. Ke B, Li C, Shang H. Sex hormones and risk of epilepsy: a bidirectional Mendelian randomization study. Front Mol Neurosci. 2023;16:1153907. https://doi.org/10.3389/fnmol.2023.1153907.
79. Herzog AG, Blum AS, Farina EL, Maestri XE, Newman J, Garcia E, et al. Valproate and lamotrigine level variation with menstrual cycle phase and oral contraceptive use. Neurology. 2009;72(10):911–4. https://doi.org/10.1212/01.wnl.0000344167.78102.f0.
80. Löscher W. Concentration of metabolites of valproic acid in plasma of epileptic patients. Epilepsia. 1981;22(2):169–78. https://doi.org/10.1111/j.1528-1157.1981.tb04098.x.
81. Löscher W. Anticonvulsant activity of metabolites of valproic acid. Arch Int Pharmacodyn Ther. 1981;249(1):158–63.
82. Löscher W, Potschka H. Drug resistance in brain diseases and the role of drug efflux transporters. Nat Rev Neurosci. 2005;6(8):591–602. https://doi.org/10.1038/nrn1728.
83. Baltes S, Fedrowitz M, Tortós CL, Potschka H, Löscher W. Valproic acid is not a substrate for P-glycoprotein or multidrug resistance proteins 1 and 2 in a number of in vitro and in vivo transport assays. J Pharmacol Exp Ther. 2007;320(1):331–43. https://doi.org/10.1124/jpet.106.102491.
84. Loscher W. Basic pharmacology of valproate: a review after 35 years of clinical use for the treatment of epilepsy. CNS Drugs. 2002;16(10):669–94. https://doi.org/10.2165/00023210-200216100-00003.
85. Rogawski MA, Loscher W. The neurobiology of antiepileptic drugs. Nat Rev Neurosci. 2004;5(7):553–64. https://doi.org/10.1038/nrn1430.
86. Löscher W, Potschka H, Sisodiya SM, Vezzani A. Drug resistance in epilepsy: clinical impact, potential mechanisms, and new innovative treatment options. Pharmacol Rev. 2020;72(3):60. https://doi.org/10.1124/pr.120.019539.
87. Schmidt D, Löscher W. Drug resistance in epilepsy: Putative neurobiologic and clinical mechanisms. Epilepsia. 2005;46(6):858–77. https://doi.org/10.1111/j.1528-1167.2005.54904.x.
88. Wang Y, Li Y, Wang G, Lu J, Li Z. Overexpression of Homer1b/c induces valproic acid resistance in epilepsy. CNS Neurosci Ther. 2023;29(1):331–43. https://doi.org/10.1111/cns.14008.

Authors and Affiliations

Dorothee Kasteleijn-Nolst Trenité[1,2] · Ronald C. Reed[3] · Alessandro Ferretti[4] · Anteo Di Napoli[5] · Pasquale Parisi[4]

✉ Ronald C. Reed
rreed@wmcarey.edu; RReedCWRU@aol.com

1 Department of Neurosurgery and Epilepsy, University Medical Center, Utrecht University, Utrecht, the Netherlands

2 Neuroscience, Mental Health and Sense Organs (NESMOS) Department, Faculty of Medicine and Psychology, Sapienza University of Rome, Rome, Italy

3 School of Pharmacy, William Carey University, Biloxi, MS, USA

4 Pediatrics Unit, Neuroscience, Mental Health and Sense Organs (NESMOS) Department, Faculty of Medicine and Psychology, Sapienza University of Rome, Rome, Italy

5 Epidemiology Unit, Migration and Poverty (INMP), National Institute for Health, Rome, Italy