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Fluctuating and constant valproate administration gives equivalent seizure control in rats with genetic and acquired epilepsy

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ABSTRACT

Purpose: Controlled-release formulations of Valproate (VPA) reduce side effects by minimizing peak plasma VPA concentrations in patients with epilepsy. However, the impact of this on anti-seizure efficacy has not been thoroughly explored. Here the pharmacokinetics and pharmacodynamics of chronic intermittent (consequently, peak VPA concentrations) and continuous VPA administration were directly compared in two rat models of epilepsy.

Methods: Genetic Absence Epilepsy Rats from Strasbourg (GAERS) received a single acute bolus of VPA (100 mg/kg intravenously) combined with electroencephalography (EEG) and/or blood sampling for 180 min post-injection. GAERS and epileptic rats post-kainic acid-induced status epilepticus were chronically infused intravenously (3–5 days, respectively) with (i) saline followed by in random order (ii) intermittent and (iii) continuous VPA (42 mg/kg/h), separated by two days of wash-out. Seizures were quantified using video-EEG monitoring and VPA levels measured in brain, cerebrospinal fluid and plasma.

Results: Following acute VPA administration seizure suppression in GAERS persisted after plasma VPA levels became very low. Chronic intermittent and continuous VPA significantly suppressed seizures in both models ($p < 0.01$) with no difference between administration regimens. In GAERS, the pattern of seizure suppression during intermittent treatment was constant, in contrast to the fluctuating VPA plasma and brain levels. There was discordance between the temporal pattern of plasma, brain VPA levels and seizure suppression efficacy in GAERS.

Conclusion: Administration regimes that result in fluctuating VPA blood levels achieve equivalent sustained seizure suppression as those that maintain steady mid-range concentrations.

1. Introduction

Valproate (valproic acid, VPA) is one of the most widely used anti-epileptic drugs (AED) in clinical practice world-wide, with a broad-spectrum of activity across different seizure types in both children and adults.[1] It is generally regarded as the drug of choice for the control of all types of generalised epilepsy syndromes,[2] but is also effective in the treatment of partial seizures, with or without secondary generalisation, and acutely in status epilepticus.[3] However, concerns around its tolerability (e.g. sedation and weight gain),[4,5] teratogenicity in pregnant women[6,7] and potential for serious organ toxicity (e.g. hepatotoxicity and pancreatic toxicity)[8,9] influence the use of VPA in clinical practice.

Minimizing peak concentrations by administering VPA in an extended-release preparation, resulting in less fluctuations in plasma concentrations[10] has been advocated as an approach to reduce side effects.[11] Indeed, extended-release divalproex sodium, especially engineered for slow release over 24 h, significantly decreased side effects in epilepsy patients.[12] Another advantage of sustained release VPA formulations over conventional formulations is the reduced dosing frequency required (i.e. once daily), potentially aiding patient compliance.[13] In animal models of embryo toxicity testing, high peak plasma levels (i.e. Cmax) of VPA, but not the total daily dose, correlated with the incidence of neural tube defects.[14]

'However, the impact on seizure control of the higher peak and lower trough serum levels of VPA that result from traditional

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as compared to sustained release formulations has received little specific scientific study. Lower trough levels may potentially be associated with an increased risk of breakthrough seizures. Conversely, it has been suggested that peak VPA levels may be necessary to achieve optimal anti-epileptic efficacy.[11,15] The primary mechanisms by which VPA exerts its anti-seizure effects are still uncertain. *In vitro* neurophysiological experiments show that VPA has an early effect to inhibit cellular excitability which is exerted from the extracellular side of the neuronal membrane, and a late effect resulting from intracellular actions of VPA which is delayed at least in part because of slow penetration of VPA into neurons.[16,17] The latter effect might benefit from high peak VPA levels promoting diffusion into neurons.[18] However, a randomised control study in humans using divalproex extended-release formula, indicates that there is no loss of seizure control.[19] In animals, it has been demonstrated that conventional VPA administration (dose resulting in peak concentrations) is not necessary to achieve anticonvulsant action in the pentylenetetrazol (PTZ)-infusion seizure threshold model.[18] Furthermore, continuous administration of VPA resulted in higher tolerability and fewer side effects.

In the experiments reported here, two rat models displaying spontaneous seizures were used in which clear VPA antiepileptic effects have been demonstrated previously.[20–23] Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and the status epilepticus (SE) model are both well validated models of genetic generalised[20] and acquired limbic epilepsy,[24] respectively. Testing efficacy in models of these distinct forms of epilepsy is relevant because AEDs may have differential effects on different types of epilepsy,[25] and these models represent the two most common broad groups of human epilepsies. Of particular importance for this study, GAERS have a high seizure frequency (on average every 1–2 min) which enables the temporal relationship between plasma VPA levels and anti-seizure efficacy to be investigated. A better understanding of the effect of plasma VPA fluctuations on brain penetration and anti-seizure efficacy has implications for the design and delivery of oral formulations in clinical practice. Therefore, the aims of this study were to compare intermittent (with attendant peak VPA concentrations) versus constant intravenous (i.v.) VPA administration in two rodent models of epilepsy for: (i) anti-seizure efficacy, (ii) plasma and cerebrospinal fluid (CSF) concentrations and (iii) brain penetration.

2. Methods

2.1. Animals

Rats were bred at the Ludwig Institute for Cancer Research and the Biological Research Facility (the Royal Melbourne Hospital). Temperature was controlled to a constant 22 °C and animals were put on a 12 h light/dark cycle with *ad libitum* food and water. One week before experimentation rats were individually housed. All experiments were approved by the Melbourne Health Animal Ethics Committee (project 2006.035), the University of Melbourne Animal Ethics Committee and followed the Principles of Laboratory Animal Care (NIH publication No. 86-23 revised 1996).

Twenty one male (338 ± 5 g) and 7 female (225 ± 16 g) 4 month old GAERS and 22 male non-epileptic control (NEC) rats 9–10 weeks old (391 ± 7 g) were used. All NEC rats were treated with repeated low-dose injections of KA (5–10 mg/kg, OPIKA-1™ Ocean Produce International, Shelburne, Canada) until SE was induced.[26] SE was defined as a period of continuous seizures sustained for 4 h and terminated with diazepam (Mayne Pharma, Adelaide, Australia) (4 mg/kg) as previously described.[27,28]

2.2. Study design

The study consisted of three experimental parts: (i) an acute single bolus study in GAERS, (ii) a chronic infusion study in GAERS and (iii) a chronic infusion study in post-KA SE rats.

(i) The acute single bolus administration study in GAERS: 12 GAERS received a single dose of VPA (100 mg/kg) i.v., via a previously implanted jugular vein cannula, with the animal moving freely in its home case. This dose was chosen on the basis of previous studies that had demonstrated significant seizure suppression with the use of this dose as an acute bolus injection in GAERS.[29] EEG was recorded for 1 h before and 3 h after each bolus using Compumedics ProFusion EEG acquisition software (Melbourne, Australia). Blood was taken (0.1 mL) from 5 GAERS: 2, 5, 30, 60, 120, 180 min post-VPA administration for analysis of VPA plasma levels.

(ii) The chronic infusion study in GAERS (Fig. 1): 16 rats (9 male, 7 female) were first continuously infused for 4 days with 0.9% saline i.v. (1 mL/h) followed by in random order (cross-over) two serial VPA treatment regimens each 3 days long: intermittent infusions (42 mg/kg/1 mL over 4 min every hour) or continuous VPA infusion (42 mg/kg/1 mL/h) separated by a 2 day wash-out period of saline (1 mL/h). EEG was acquired on day 3 of each treatment regimen for 24 h. The last 24 h of the chronic infusion treatment periods were chosen for the EEG analysis in the GAERS study because this represented a time period in which steady-state equilibrium plasma levels are reached, and best represented the situation of patients chronically taking VPA. To investigate long-lasting effects of chronic VPA treatment, EEG was also acquired for the first 24 h of the wash-out period in eight animals (four had received the continuous VPA regimen and four the intermittent regimen).

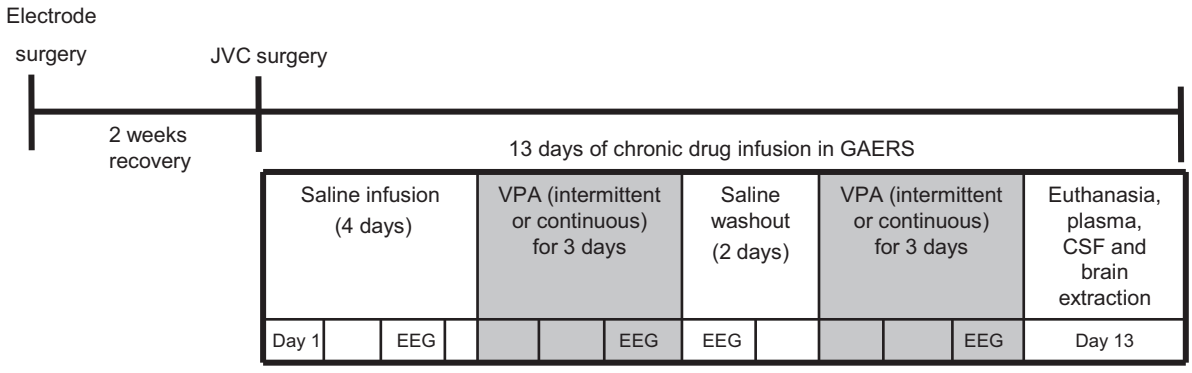

Fig. 1. Overview of the experimental design for GAERS. Surgery, chronic saline and VPA administration.

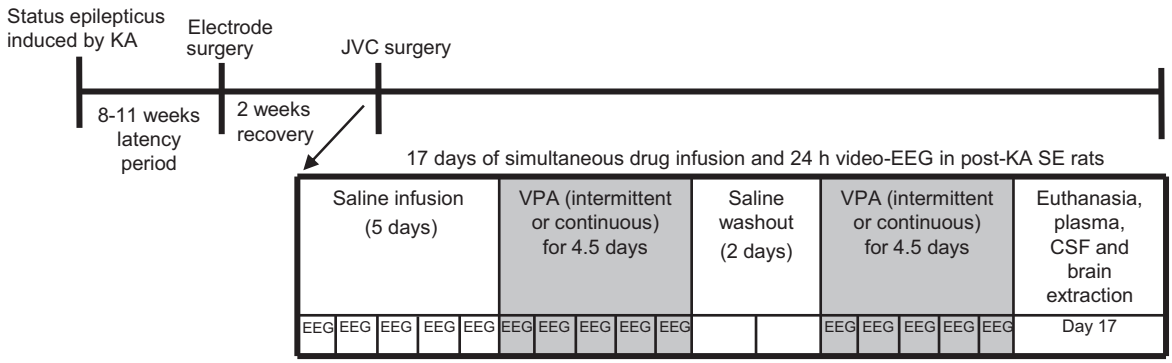

Fig. 2. Overview of the experimental design for post-KA status epilepticus rats. Surgery, chronic saline and VPA administration timeline.

(iii) The chronic infusion in post-KA SE rats (Fig. 2): Rats underwent a similar protocol consisting of VPA infusion and continuous 24 h video-EEG monitoring. Twenty-two animals received saline for 5 days, and intermittent and continuous VPA for 4.5 days each (in a randomised order, cross-over). Twelve animals did not display spontaneous seizures during the saline period and one animal did not complete the study due to being unwell; these animals were excluded from the analysis

A blood sample was taken in a subgroup of rats at the end of the washout period.

2.3. Surgery

EEG electrodes were implanted under ketamine/xylazine anaesthesia in a stereotaxic frame as described previously.[28] During surgery, heart rate and blood oxygen levels were measured. GAERS received 6 epidural electrodes and post-KA SE rats received 4 epidural electrodes and some also a bipolar electrode into the left hippocampus (coordinates from bregma: anterioposterior – 4.6 mm, mediolateral – 5.8 mm, dorsoventral – 7 mm). A 9-pin ABS plug (GS09PLG-220, Ginder Scientific, Canada)[30] was used to fix the electrodes and to hold the PVC jugular vein cannula (JVC). Saline (0.9% NaCl, 1 mL, s.c.) was administered to prevent dehydration and carprofen (5 mg/kg, Pfizer, Australia) was injected i.p. for pain relief at the end of each surgery. Animals were returned to their home cages for recovery.

Two weeks later a JVC (internal diameter = 0.8 mm, outside diameter = 1.2 mm, Microtube Extrusions Pty Ltd, North Rocks, Australia) was implanted under isoflurane anaesthesia as described previously.[28] The EEG/infusion cable and head cap were locked together via a Delrin Ring Nut (GS09BDN-220, Ginder Scientific, Nepean, Canada).[28] Carprofen (5 mg/kg, Pfizer, West Ryde, Australia) was injected i.p. at the end of surgery for pain relief. The animals were placed into their experimental cage to recover and saline infusion commenced within 1–3 h post-surgery.

2.4. Drug delivery

Valproate in the form of valproic acid sodium salt (Sigma–Aldrich, St. Louis, USA) was dissolved in saline (0.9% NaCl, 5 mL/kg) at two different doses. The concentration of the single acute bolus study was 100 mg/kg and for the chronic infusion study 42 mg/kg/h containing 4 IU/mL Heparin (Heparin Sodium DBL, Hameln Pharmaceuticals GmbH, Hameln, Germany). The latter dose has previously been administered i.v. and was found to produce therapeutic serum levels of VPA (600–1800 μmol/L).[31]

Drug delivery was carried out via an EEG/infusion cable (made in house) connected through an EEG and fluid swivel, allowing free movement, to a 32 channel video-EEG system (Compumedics Limited, Melbourne, Australia) and a multi-syringe programmable pump (KDS 230, Walker Scientific, Wangara, Australia) as previously described.[28] To assist in preventing infusion lines clotting, animals were given a daily 0.1 mL bolus of heparinised saline (20 IU/mL). Any leakages or blockages were fixed if possible, and if not, the rat was removed from the study for the treatment arm they did not complete and euthanased (one GAERS received intermittent only and two others continuous infusion only).

2.5. Plasma, CSF and brain VPA levels

At the end of the chronic infusions animals were euthanased 5 ($n = 4$), 30 ($n = 5$) and 55 ($n = 5$) min post-bolus or during continuous infusion ($n = 13$) using a Lethabarb overdose (325 mg pentobarbitone sodium, i.p., Virbac Animal Health, Carros Cedex, France). When rats were fully anaesthetised a cerebral spinal fluid (CSF) sample (0.1 mL) was extracted from the cisterna magna area, which was successful in most animals, some samples were contaminated with blood and thus discarded. Blood (1 mL) was sampled from the heart to investigate VPA plasma levels and was centrifuged at 8000 rpm for 5 min. Plasma VPA analysis was carried out on an Abbott TDX system (analytical range: 5–1040 μmol/L using Fluorescence Polarization Immunoassay) (Abbott Diagnostics, Abbott Park, USA).

Subsequently the rat brain was rapidly removed and dissected into four regions: hippocampus, thalamus, cortex and the remaining areas. Brain regions were homogenised in a 1:1 ratio of brain weight to distilled water (to include intracellular and extracellular VPA in the results), except for the hippocampus, which was diluted in a 1:3 ratio because of the small volume of tissue compared with the other brain regions. Brain homogenates were centrifuged at 12,000 rpm for 10 min at 4 °C and the supernatant removed for VPA analysis with the Abbott TDX system.

2.6. EEG data processing and analysis

For the acute study, seizures during the bolus study in GAERS were quantified by reviewing the 4 h EEG recording using the Mighty EDF® EEG viewing software (version 1.3.3, University of Ghent, Ghent, Belgium) and manually marking the start and the end of the seizure. The comparison of % time in seizure pre- versus post-treatment in GAERS was calculated by averaging four 15 min blocks in the hour prior to the drug injection, then comparing each subsequent 15 min block post-treatment to this baseline value.

The 24 h EEG recordings from the chronic GAERS study were analysed using a semi-automated SWD detection program (SWDfinder, Radboud Universiteit, Nijmegen, The Netherlands), which we have validated to detect SWDs with high sensitivity (100%) and accuracy (95%) versus manual seizure identification. The software

highlights automatically detected seizures, which were subsequently manually confirmed and start/end of seizures was adjusted if required.

Data from the chronic infusion is represented as % time in seizure, total number of seizures and average seizure duration. In addition, 24 h EEG data recorded during intermittent VPA treatment was separated into four 15 min blocks (0–15 min, 15–30 min, 30–45 min and 45–60 min post-bolus) and expressed as average % time in seizure. Eight animals had a 24 h EEG recording on the first day of the wash-out period. EEG data acquired during the washout period was separated into six 4 h time blocks post-cessation of VPA.

For the post-KA SE rats, spontaneous convulsive seizures were quantified by manual review of the continuous synchronised video-EEG recordings (ProFusion EEG v3.7, Compumedics Limited, Melbourne, Australia). As animals were mainly displaying convulsive seizure Class IV–V, data were expressed as number of Class IV and V convulsive seizures according to the scale of Racine[32] per day per rat. All analysis was carried out blinded.

2.7. Statistical analysis

The EEG data was not normally distributed and therefore was analysed using non-parametric tests. For the GAERS study, no differences were found between male and female rats; therefore, the data of both genders was combined. The bolus study was analysed using a one-way ANOVA Friedman test followed by a post hoc Dunns test for comparison of each 15 min time block of % time in seizure post-VPA. A Friedman test was used for comparison of absence seizures between saline, intermittent and continuous VPA treatment, followed by a post hoc Wilcoxon signed rank (1-tail for saline versus treatment and 2-tailed for intermittent versus continuous). A Friedman test was used followed by a post hoc Dunns test on convulsive seizures in post-KA SE animals and to compare % time in seizure during four different time blocks (15 min) post-hourly VPA infusion in the intermittent VPA treated GAERS. A Wilcoxon signed rank test was used to compare % time in seizure of six 4 h time blocks post-VPA treatment during the first day of the saline wash-out period between corresponding time points of saline and VPA infusion conditions.

VPA levels in plasma, CSF and brain regions were normally distributed and analysed with a one-way ANOVA followed by a planned comparisons Bonferroni test.

Data are presented as mean ± S.E.M. for the parametric tests. Significance was set at $p < 0.05$.

3. Results

3.1. Acute single bolus VPA administration in GAERS

Compared to baseline, seizures were significantly suppressed up to 165 min post-VPA bolus administration compared to baseline, although this did not attain statistical significance for the 15 min time blocks at 90 and 150 min (Fig. 3A). Corresponding VPA plasma levels 2, 5, 30, 60, 120, 180 min are plotted in Fig. 3B. By 120 min post-bolus the plasma VPA levels had declined to very low levels (61 + 13 μmol/L, i.e. 1.7% of peak levels). It is

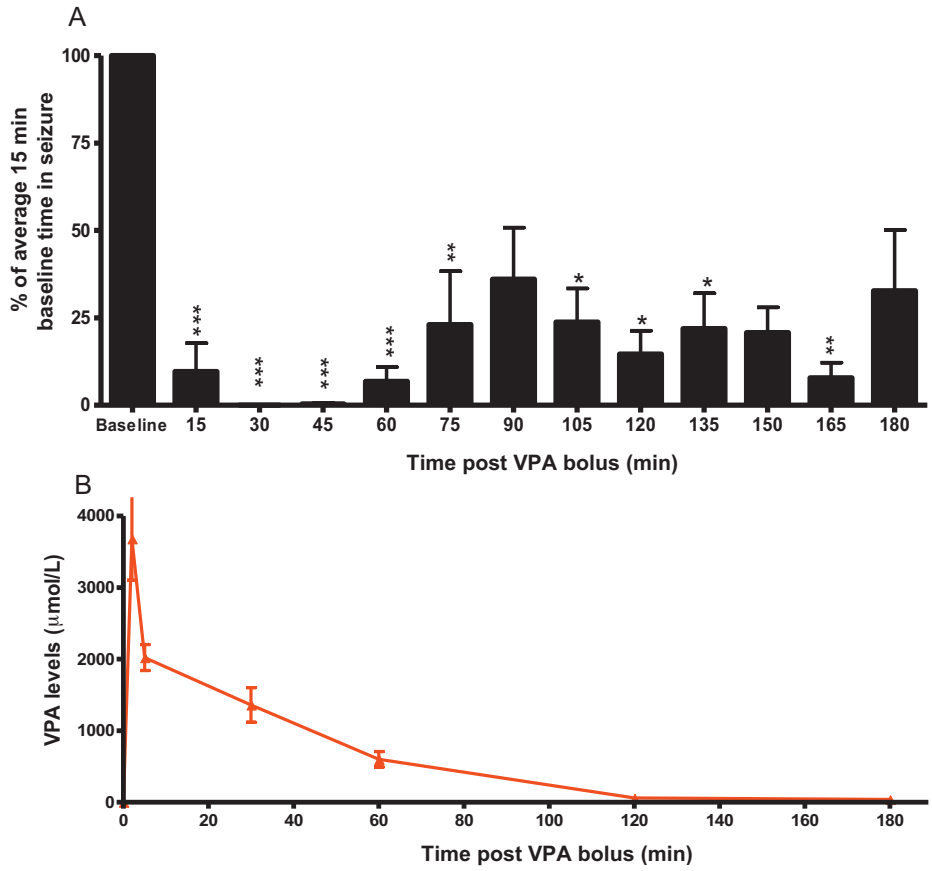

Fig. 3. Temporal seizure suppression and VPA plasma levels after a bolus of 100 mg/kg VPA. (A) % of average 15 min baseline time in seizure $n = 12$. Friedman test with a post hoc Dunns test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ all compared to the corresponding baseline period. (B) VPA plasma levels (μmol/L) post 100 mg/kg bolus of VPA $n = 5$. Data are expressed as mean ± S.E.M.

noteworthy that seizure suppression persisted for at least another 45 min following this time point.

3.2. Chronic intermittent and continuous VPA infusion in GAERS

Both intermittent and continuous VPA treatment arms resulted in significantly suppressed % time in seizure (−58% and −52%, respectively, $p < 0.01$, Fig. 4A), mainly due to a decrease in seizure number ($p < 0.01$, Fig. 4B), while the seizure duration was not significantly affected (Fig. 4C). There was no significant difference between intermittent and continuous treatment regimens in % time in seizure, number of seizures, or seizure duration.

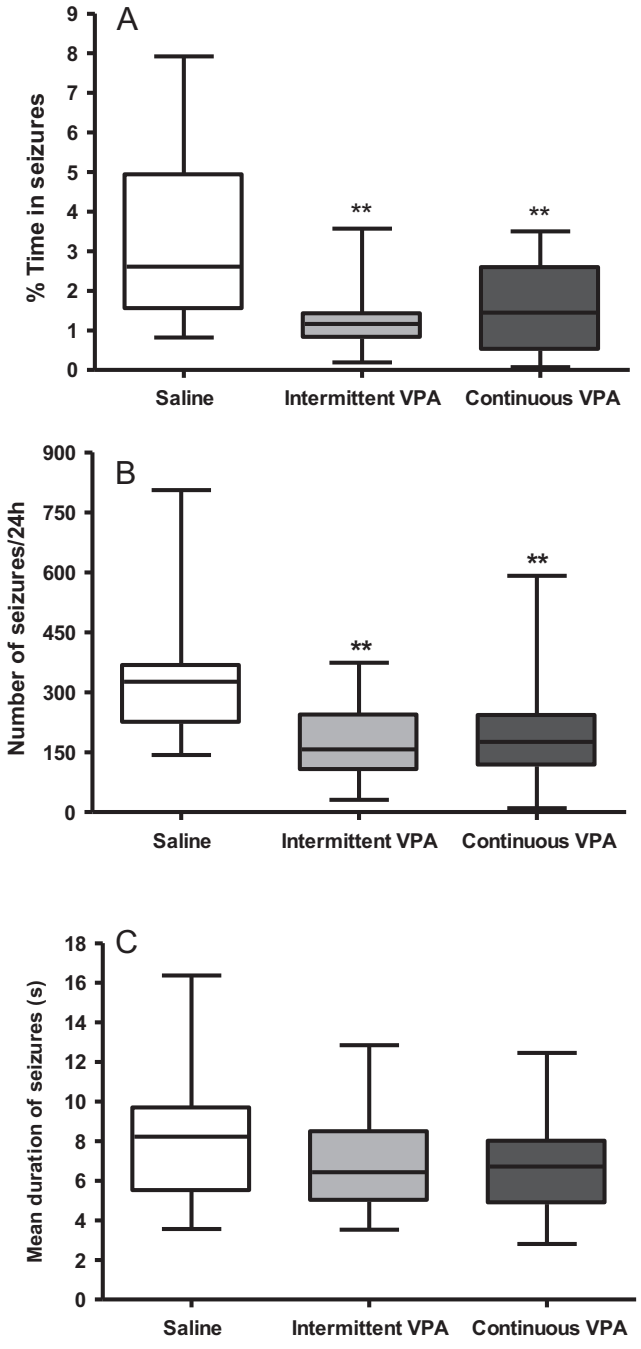

Fig. 4. Seizure expression during chronic infusions of saline, intermittent VPA and continuous VPA in GAERS: % time in seizures, number of seizures per 24 h and mean duration of seizures. Box plot indicates the median with lower and upper quartile ranges and sample minimum and maximum values. A Friedman test with a post hoc Wilcoxon signed rank (1-tail for saline versus treatment and 2-tailed for intermittent versus continuous) was used. $^{**}p < 0.01$. Saline $n = 16$, continuous VPA $n = 15$, intermittent VPA $n = 14$.

Further analysis of the intermittent treatment regimen into 15 min time blocks demonstrated that seizure suppression did not significantly vary between any of the time blocks in the hour post the bolus (Fig. 5).

No order of treatment effect was observed in the intermittent and fluctuating treatment periods for any of the seizure characteristics ($p > 0.05$, data not shown).

EEG-monitoring during the first 24 h of the wash-out period demonstrated that seizures were being suppressed up until 20 h post-VPA when compared to saline, with the time blocks 12–16 h and 16–20 h reaching significance ($p < 0.05$) when a Wilcoxon signed rank test was applied to individual time blocks (Fig. 6).

3.3. Chronic intermittent and continuous VPA infusion in post-KA SE rats

Freedom from convulsive seizures (Class IV or V) was achieved in 9/9 post-KA animals during the chronic intermittent VPA infusion versus 7/9 during the continuous VPA infusion. Both chronic VPA treatment regimens were effective in suppressing these seizures compared to the saline infusion arm ($p < 0.01$, Fig. 7).

There was no significant difference between intermittent and continuous treatment or between the first or second VPA treatment periods in this cross-over study. Blood samples from the end of the washout period contained no detectable VPA levels.

3.4. Brain, plasma and CSF VPA levels following intermittent and continuous infusions

There was no difference observed between the rat models, hence results were combined for this analysis. VPA thalamic and hippocampal brain uptake, CSF and plasma concentrations were significantly different between the time points (ANOVA, $p < 0.001$, $p < 0.01$, $p < 0.001$, $p < 0.001$, respectively) and approached significance for the cortex (ANOVA, $p = 0.08$) as shown in Fig. 8.

Post hoc Bonferroni analysis demonstrated that peak VPA levels (5 min post-bolus) were significantly higher than trough VPA levels (55 min post-bolus administration) for thalamus, hippocampus, CSF and plasma (Fig. 8), with approximately a four-, five- and three-fold difference between peak and trough brain levels, CSF and plasma, respectively. CSF and plasma levels were significantly higher at 5 min post-bolus administration compared to 30 min post-bolus and continuous administration (Fig. 8). This confirms that the intermittent protocol truly resulted in fluctuating VPA concentrations. There was no significant difference in VPA concentration between 30 min post-bolus and continuous VPA administration (Fig. 8).

VPA concentrations were similar for the different brain regions (Fig. 8). Plasma concentrations were around 3–4 times higher than CSF values. Plasma levels were closely correlated linearly with those of: CSF $R = 0.91$, cortex $R = 0.86$, thalamus $R = 0.87$, hippocampus $R = 0.84$, rest of brain $R = 0.88$ ($p < 0.01$, $n = 20$–27).

4. Discussion

This study evaluated the pharmacokinetics and pharmacodynamics of acute and chronic VPA administration in two animal models of epilepsy. We observed two major findings: (a) in the intermittent delivery protocols, both acute and chronic, a temporal discordance between VPA plasma levels and amount of seizure suppression (measured in the GAERS) occurred, and (b) an equivalent seizure reduction, as compared to saline infusion, was achieved in both the intermittent and continuous chronic VPA

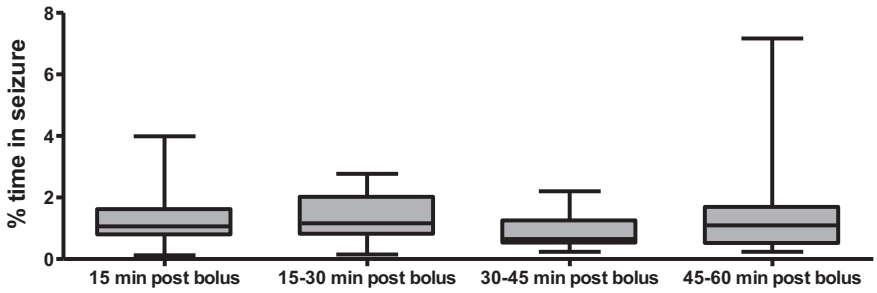

Fig. 5. Average seizure expression during 15 min time blocks of hourly VPA treatment in GAERS (averaged over 24 hours). Box plot indicates the median with lower and upper quartile ranges and sample minimum and maximum values. With application of a Friedman test, no significant difference was found between groups ($n = 13$).

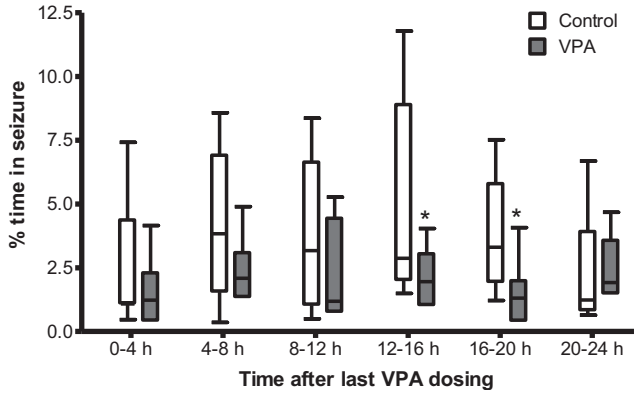

Fig. 6. % Time in seizure for sequential 4 h time blocks after cessation of chronic VPA infusion (4 continuous and 4 intermittent rats) during the first 24 h of saline washout in GAERS (Wilcoxon signed rank test) versus the same time blocks following saline infusion ($n = 8$). $^{*}p < 0.05$. Box plot indicates the median with lower and upper quartile ranges and sample minimum and maximum values.

delivery protocols in the animal models. The results demonstrate that a VPA administration regimen resulting in fluctuating (high peak) total VPA levels does not produce significantly different seizure control than a regimen that results in constant, relatively flat blood levels over a dosing interval. This indicates that repeated high peak plasma VPA levels are not necessary for the optimal anti-seizure effect, and that the trough periods do not result in significant breakthrough seizures. The likely explanation for the *apparent* lack of importance of the VPA trough is the observation that there is a temporal discordance between the blood levels and the anti-seizure effects, indicating that VPA is, in at least part, acting by more prolonged cellular mechanisms. Alternatively, the VPA troughs were still above the threshold to provide maximal anti-seizure efficacy.

No significant differences were found in the amount of seizure suppression between any of the four averaged 15 min time blocks post-repeated intermittent VPA boli in GAERS, despite a three- to five-fold difference between peak and trough plasma, CSF and brain levels (Fig. 8). Brain and CSF VPA levels correlated tightly with plasma levels during intermittent (fluctuating) and continuous VPA administration. This indicates that the discordance between the plasma levels and seizure suppression was not due to delayed brain entry of the VPA. Furthermore, seizures were significantly suppressed up to 20 h following the cessation of the chronic infusion compared with saline only treatment. Following a single acute injection the seizures were suppressed for at least 165 min post-VPA administration, well after the VPA plasma concentrations had declined to negligible levels (i.e. <1.7% of peak levels 120 min post-injection). This phenomenon is sometimes referred to as the 'carry over effect', and our results provide support for this experimental[33,34] and clinical observation.[35] Nau and Loscher first described the presence of a metabolite (2-propyl-2-pentenoic acid) which persists for at least 8 h after VPA administration in mice, correlating well with the carry-over effect.[33] In rats this metabolite was shown to accumulate in the brain during prolonged treatment.[36] Although potentially an accumulation of anti-convulsant metabolite in the brain could have biased our cross-over study, we did not find a treatment order effect in this randomised study. This suggests that the effects of these anti-convulsant metabolites had largely worn off by the start of the second treatment period.

Previous studies have also shown that there is a late intracellular effect of VPA after slow penetration into the cells synergistic to an acute rapid extracellular effect on neuronal membranes.[16,17] The

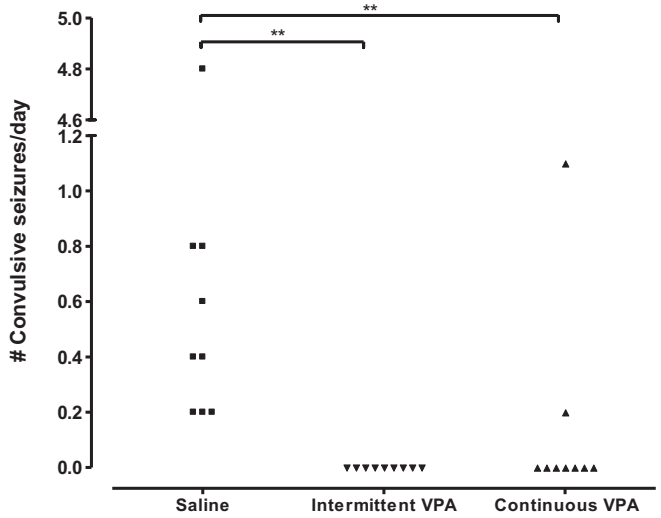

Fig. 7. The mean number (#) of convulsive seizures per day during chronic saline, intermittent and continuous VPA infusions in post-KA SE rats ($n = 9$). Friedman test with a post hoc Dunns test. $^{**}p < 0.01$. Data is shown for individual animals.

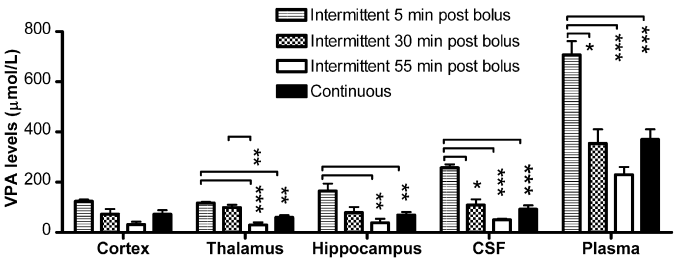

Fig. 8. VPA levels in brain structures, CSF and plasma during intermittent (5, 30 and 55 min after bolus infusion) and continuous VPA treatment. One-way ANOVA followed by a planned comparison Bonferroni test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; 5 min post-bolus, $n = 4$, 30 min post-bolus, $n = 5$, 55 min post-bolus, $n = 5$, continuous, $n = 13$. Data are expressed as mean ± S.E.M.

late intracellular effect is proposed to strongly contribute to the mechanism of action of VPA.[16] Indeed, VPA is known to activate several secondary messenger systems.[37,38] For example, neuropeptide Y (NPY), a neuropeptide with anti-epileptic properties against experimental focal[39] and generalised seizures,[40,41] has been shown to be increased in the brain after 3 days of VPA treatment, but not after acute treatment.[37] Additionally, chronic VPA administration is now known to enhance intracellular demethylase activity through its effects on histone acetylation resulting in long-lasting epigenetic changes in gene expression.[42,43]

This study is unique in that it has investigated the relationship between the pharmacokinetics (plasma, CSF and brain VPA levels) and pharmacodynamics (seizure suppression) of different VPA administration regimes in spontaneously seizing epileptic rats. Previous studies have used acute provoked seizures in otherwise non-epileptic animals,[18] which are likely to have less relevance for the human chronic epileptic condition. Studies using chronic epilepsy models are more labour intensive as they require long-term monitoring and infusion equipment.[28] The GAERS model is ideally suited for the study of pharmacokinetic-pharmacodynamics of drugs as the animals express frequent spontaneous generalised seizures, providing the temporal resolution to allow investigation of the effect of rapid changes in drug blood levels on seizure suppression. The post-KA SE model displays less frequent seizures; daily to weekly seizures compared to every 1–2 min on average in GAERS. Therefore, a longer and continuous 5 day video-EEG monitoring period was required in the post-KA SE model. This model does not allow the high temporal resolution of drug-seizure effects that enabled a close examination of the pharmacokinetic-pharmacodynamic relationships in the GAERS, but it was important to study as the anti-seizure efficacy of VPA may be less for focal limbic seizures.[1,44]

In humans, VPA is most commonly administered twice a day, with one dose roughly every half life (9–20 h).[45] In rats, VPA half-life is variable and dose dependent.[38] In addition, PK/PD relationship does not seem to be linear as exemplified by the acute bolus study in which 100 mg/kg resulted in at least >60 min seizure suppression, while a biological half-life of 37 min was measured. In the current study we chose to administer intermittently on an hourly basis and in order to keep the total daily dose the same in both chronic treatment arms (i.e. 1008 mg/kg per day), 42 mg/kg/h was administered to the intermittent group. A previous study by Arens and Pollack[31] showed sustained therapeutic plasma levels by hourly continuous infusion of 42 mg/kg of VPA. The longer time between the administration of VPA doses (e.g. 8 h) that has been used in other studies,[18] would result in prolonged periods of low VPA levels in the intermittent infusion group which could bias the results. On the other hand, we did not opt for the usual very high doses of VPA (around 200 mg/kg) used previously,[38] in order to have the dynamic range to be able to pick up effects of fluctuations in VPA levels on seizure suppression. The 3.5-fold degree of difference in peak-trough total plasma VPA concentrations observed for our chosen chronic intermittent i.v. regimen is greater than the almost 2-fold peak-trough difference observed to occur for an oral immediate-release (enteric-coated, delayed-release) divalproex sodium tablet formulation in a every 8-h dosing regimen commonly used clinically[10] and still more than the approximate average 2.4-fold peak-trough difference simulated to occur for the same preparation when taken every 24 h.[46] Accordingly, the peak VPA levels achieved in our study were more than sufficient to answer our question as to the contribution of peak plasma VPA (versus average concentration) to anti-seizure efficacy.

A previous study had compared intermittent and continuous VPA administration in the acute PTZ threshold rat model.[18] Continuous VPA treatment (600 mg/kg/day) significantly decreased PTZ seizure threshold, but bolus (200 mg/kg) followed by infusion (400 mg/kg/day) or three bolus injections a day (200 mg/kg) resulted in better seizure suppression. The apparent conflict with the results of our study could be explained by the PTZ threshold being tested 15 min after bolus injection (200 mg/kg), at which time significantly higher VPA plasma levels would be expected compared to the continuous infusion. Even though we did not find in our study that VPA levels correlated well with the degree of seizure suppression, the differences between the levels would have been substantially greater in this previous study than in ours given the higher dose bolus (200 versus 42 mg/kg) and lower dose constant infusion (25 versus 42 mg/kg) used. It is also possible that the difference in the findings represents an inherent difference in seizure susceptibility between the chemoconvulsant and genetic animal models used in the two studies.

In conclusion this study demonstrated, in truly epileptic animal models manifesting spontaneous generalised or focal seizures, that peak VPA concentrations are not required for optimal anti-seizure efficacy, with the maintenance of steady mid-range concentrations being just as potent. Furthermore, we found a temporal discordance between plasma, CSF and brain VPA levels (pharmacokinetics) and anti-seizure efficacy (pharmacodynamics) with intermittent VPA administration. These results have clinical implications, specifically regarding the use of controlled release formulations that provide less fluctuation in blood levels.

5. Conflict of interest statement

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References

1. Perucca E. Pharmacological and therapeutic properties of valproate: a summary after 35 years of clinical experience. *CNS Drugs* 2002;**16**:695–714.
2. Marson AG, Al-Kharusi AM, Alwaidh M, Appleton R, Baker GA, Chadwick DW, et al. The SANAD study of effectiveness of valproate, lamotrigine, or topiramate for generalised and unclassifiable epilepsy: an unblinded randomised controlled trial. *Lancet* 2007;**369**:1016–26.
3. Giroud M, Dumas R. Treatment of status epilepticus by sodium valproate. *Neurophysiol Clin* 1988;**18**:21–32.
4. Davis R, Peters DH, McTavish D. Valproic acid. A reappraisal of its pharmacological properties and clinical efficacy in epilepsy. *Drugs* 1994;**47**:332–72.
5. Bowden CL, Singh V. Valproate in bipolar disorder: 2000 onwards. *Acta Psychiatr Scand Suppl* 2005:13–20.
6. Kultima K, Nystrom AM, Scholz B, Gustafson AL, Dencker L, Stigson M. Valproic acid teratogenicity: a toxicogenomics approach. *Environ Health Perspect* 2004;**112**:1225–35.
7. Vajda FJ, O'Brien TJ, Hitchcock A, Graham J, Cook M, Lander C, et al. Critical relationship between sodium valproate dose and human teratogenicity: results of the Australian register of anti-epileptic drugs in pregnancy. *J Clin Neurosci* 2004;**11**:854–8.
8. Grillo MP, Chiellini G, Tonelli M, Benet LZ. Effect of alpha-fluorination of valproic acid on valproyl-S-acyl-CoA formation in vivo in rats. *Drug Metab Dispos* 2001;**29**:1210–5.
9. Neuman MG, Shear NH, Jacobson-Brown PM, Katz GG, Neilson HK, Malkiewicz IM, et al. CYP2E1-mediated modulation of valproic acid-induced hepatocytotoxicity. *Clin Biochem* 2001;**34**:211–8.
10. Sommerville KW, Dutta S, Biton V, Zhang Y, Cloyd JC, Uthman B. Bioavailability of a divalproex extended-release formulation versus the conventional divalproex formulation in adult patients receiving enzyme-inducing antiepileptic drugs. *Clin Drug Invest* 2003;**23**:661–70.

11. Pellock JM, Smith MC, Cloyd JC, Uthman B, Wilder BJ. Extended-release formulations: simplifying strategies in the management of antiepileptic drug therapy. *Epilepsy Behav* 2004;**5**:301–7.
12. Smith MC, Centorrino F, Welge JA, Collins MA. Clinical comparison of extended-release divalproex versus delayed-release divalproex: pooled data analyses from nine trials. *Epilepsy Behav* 2004;**5**:746–51.
13. Herranz JL, Arteaga R, Adin J, Armijo JA. Conventional and sustained-release valproate in children with newly diagnosed epilepsy: a randomized and crossover study comparing clinical effects, patient preference and pharmacokinetics. *Eur J Clin Pharmacol* 2006;**62**:805–15.
14. Nau H. Teratogenic valproic acid concentrations: infusion by implanted minipumps vs. conventional injection regimen in the mouse. *Toxicol Appl Pharmacol* 1985;**80**:243–50.
15. Van Duijn H, Meijer JWA, Segers JP, Gardner-Thorpe C, Janz D, Meinardi H, et al. To what extent is drug entry into brain dependent on the way of administration. Some dipropylacetic acid experiments on cats. *Antiepileptic drug monitoring*. Kent: Pitman Medical Publishing Co.; 1977.
16. Altrup U, Gerlach G, Reith H, Said MN, Speckmann EJ. Effects of valproate in a model nervous system (buccal ganglia of Helix pomatia). I. Antiepileptic actions. *Epilepsia* 1992;**33**:743–52.
17. Lucke A, Lehmenkuhler A, Altrup U, Nicholson C, Reith H, Schmitz-Moormann C, et al. Diffusion analysis of valproate and trans-2-en-valproate in agar and in cerebral cortex of the rat. *Brain Res* 1993;**631**:187–90.
18. Loscher W, Honack D. Comparison of anticonvulsant efficacy of valproate during prolonged treatment with one and three daily doses or continuous (''controlled release'') administration in a model of generalized seizures in rats. *Epilepsia* 1995;**36**:929–37.
19. Thibault M, Blume WT, Saint-Hilaire JM, Zakhari R, Sommerville KW. Divalproex extended-release versus the original divalproex tablet: results of a randomized, crossover study of well-controlled epileptic patients with primary generalized seizures. *Epilepsy Res* 2002;**50**:243–9.
20. Marescaux C, Vergnes M, Depaulis A. Genetic absence epilepsy in rats from Strasbourg – a review. *J Neural Transm Suppl* 1992;**35**:37–69.
21. Leite JP, Cavalheiro EA. Effects of conventional antiepileptic drugs in a model of spontaneous recurrent seizures in rats. *Epilepsy Res* 1995;**20**:93–104.
22. Nissinen J, Pitkanen A. Effect of antiepileptic drugs on spontaneous seizures in epileptic rats. *Epilepsy Res* 2007;**73**:181–91.
23. van Vliet EA, Edelbroek PM, Gorter JA. Improved seizure control by alternating therapy of levetiracetam and valproate in epileptic rats. *Epilepsia* 2009.
24. Morimoto K, Fahnestock M, Racine RJ. Kindling and status epilepticus models of epilepsy: rewiring the brain. *Prog Neurobiol* 2004;**73**:1–60.
25. Perucca E, Gram L, Avanzini G, Dulac O. Antiepileptic drugs as a cause of worsening seizures. *Epilepsia* 1998;**39**:5–17.
26. Hellier JL, Patrylo PR, Buckmaster PS, Dudek FE. Recurrent spontaneous motor seizures after repeated low-dose systemic treatment with kainate: assessment of a rat model of temporal lobe epilepsy. *Epilepsy Res* 1998;**31**:73–84.
27. Powell KL, Ng C, O'Brien TJ, Xu SH, Williams DA, Foote SJ, et al. Decreases in HCN mRNA expression in the hippocampus after kindling and status epilepticus in adult rats. *Epilepsia* 2008;**49**:1686–95.
28. van Raay L, Morris MJ, Reed RC, O'Brien TJ, Dedeurwaerdere S. A novel system allowing long-term simultaneous video-electroencephalography recording, drug infusion and blood sampling in rats. *J Neurosci Meth* 2009;**179**:184–90.
29. Micheletti G, Vergnes M, Marescaux C, Reis J, Depaulis A, Rumbach L, et al. Antiepileptic drug evaluation in a new animal model: spontaneous petit mal epilepsy in the rat. *Arzneimittelforschung* 1985;**35**:483–5.
30. Molino A, McIntyre DC. Another inexpensive headplug for the electrical recording and or stimulation of rats. *Physiol Behav* 1972;**9**:273–5.
31. Arens TL, Pollack GM. Nonstationary disposition of valproic acid during prolonged intravenous infusion: contributions of unbound clearance and protein binding. *Biopharm Drug Dispos* 2001;**22**:243–9.
32. Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol* 1972;**32**:281–94.
33. Nau H, Loscher W. Valproic acid: brain and plasma levels of the drug and its metabolites, anticonvulsant effects and gamma-aminobutyric acid (GABA) metabolism in the mouse. *J Pharmacol Exp Ther* 1982;**220**:654–9.
34. Loscher W, Fisher JE, Nau H, Honack D. Valproic acid in amygdala-kindled rats: alterations in anticonvulsant efficacy, adverse effects and drug and metabolite levels in various brain regions during chronic treatment. *J Pharmacol Exp Ther* 1989;**250**:1067–78.
35. Rothrock JF, Mendizabal JE. An analysis of the ''carry-over effect'' following successful short-term treatment of transformed migraine with divalproex sodium. *Headache* 2000;**40**:17–9.
36. Loscher W, Nau H. Distribution of valproic acid and its metabolites in various brain areas of dogs and rats after acute and prolonged treatment. *J Pharmacol Exp Ther* 1983;**226**:845–54.
37. Brill J, Lee M, Zhao S, Fernald RD, Huguenard JR. Chronic valproic acid treatment triggers increased neuropeptide y expression and signaling in rat nucleus reticularis thalami. *J Neurosci* 2006;**26**:6813–22.
38. Loscher W. Valproate: a reappraisal of its pharmacodynamic properties and mechanisms of action. *Prog Neurobiol* 1999;**58**:31–59.
39. Noe F, Pool AH, Nissinen J, Gobbi M, Bland R, Rizzi M, et al. Neuropeptide Y gene therapy decreases chronic spontaneous seizures in a rat model of temporal lobe epilepsy. *Brain* 2008;**131**:1506–15.
40. Morris MJ, Gannan E, Stroud LM, Beck-Sickinger AG, O'Brien TJ. Neuropeptide Y suppresses absence seizures in a genetic rat model primarily through effects on Y receptors. *Eur J Neurosci* 2007;**25**:1136–43.
41. Stroud LM, O'Brien TJ, Jupp B, Wallengren C, Morris MJ. Neuropeptide Y suppresses absence seizures in a genetic rat model. *Brain Res* 2005;**1033**:151–6.
42. Detich N, Bovenzi V, Szyf M. Valproate induces replication-independent active DNA demethylation. *J Biol Chem* 2003;**278**:27586–92.
43. Milutinovic S, D'Alessio AC, Detich N, Szyf M. Valproate induces widespread epigenetic reprogramming which involves demethylation of specific genes. *Carcinogenesis* 2007;**28**:560–71.
44. Jeavons PM, Clark JE. Sodium valproate in treatment of epilepsy. *Brit Med J* 1974;**2**:584–6.
45. Genton P. Progress in pharmaceutical development presentation with improved pharmacokinetics: a new formulation for valproate. *Acta Neurol Scand Suppl* 2005;**182**:26–32.
46. Reed RC, Dutta S, Liu W. Once-daily dosing is appropriate for extended-release divalproex over a wide dose range, but not for enteric-coated, delayed-release divalproex: evidence via computer simulations and implications for epilepsy therapy. *Epilepsy Res* 2009;**87**:260–7.