Appendix 2

Three out of our five patients (cases 1-3) showed elevated ammonia serum levels. Very high ammonia levels have previously been described in patients with VE, even with normal liver function tests [21], and it has been hypothesised that the manifestations of VE are directly related to hyperammonaemia. VPA can increase the transport of glutamine across the mitochondrial membrane in the kidney, thereby increasing the production of ammonia [8]. Thus, hyperammonaemia may be due to increased renal ammonia production [22]. On the other hand, VPA inhibits carbamoylphosphatase synthase which reduces hepatic ammonia metabolism by decreasing carnitine availability. This suppresses fatty acid beta-oxidation [8, 23]. This theory is supported by the observation that VE seems to be more frequent in patients with carnitine deficiency or with congenital urea cycle enzymatic defects [8, 23]. It has also been shown that serum free carnitine concentrations are reduced in patients with VPA-associated hyperammonaemia. Serum ammonia concentrations correlate directly with serum concentrations of VPA whilst correlating inversely with serum concentrations of carnitine [23]. Based on this observation, it has been suggested that carnitine supplementation during VPA therapy could prevent VPA-induced hepatotoxicity in high-risk patients (especially in children with epilepsy < 2 years of age, anti-epileptic drug polytherapy, ketogenic diet, poor nutritional status) [23]. However, the value of L-carnitine therapy in VE and acute VPA overdose is not clear and there are no conclusive studies on the clinical value of such a therapy in adults with VPA-induced central nervous system side-effects, e. g. in VE. Nevertheless, carnitine supplementation seems to have no particular side-effects and could, theoretically, also be of benefit to adult patients with VPA-induced hyperammonaemia.

The commonest cause of hyperammonaemia is liver disease and much of our understanding of the mechanisms of ammonia-mediated neurotoxicity come from the study of this situation. Liver disease, however, is a much more complicated state than simply hyperammonaemia, and there are other mechanisms that are relevant in liver disease that do not apply to other
causes of hyperammonaemia [24]. Hepatic encephalopathy (HE) associated with chronic liver disease is unlikely to be purely mediated through hyperammonaemia and is phenotypically different to the encephalopathy associated with VPA induced hyperammonaemia. Any conclusions drawn from studying one state therefore need to be applied to the other with caution. It is, however, very interesting that the encephalopathy seen in fulminant hepatic failure shares many characteristics with the encephalopathy seen with VPA induced hyperammonaemia. In fulminant hepatic failure, portal-systemic shunting and the other mechanisms that underlie the encephalopathy associated with chronic liver disease have not had time to develop and the situation is therefore much more similar to a purely hyperammonaemic state [24]. In this condition, the encephalopathy is associated with seizures, cerebral oedema and alterations in cerebral blood flow [24], which is similar to that seen in our patients with VPA-induced hyperammonaemia but without liver disease. However, as demonstrated by our cases, VE can also occur in the presence of normal levels of ammonia [8]. There are several possible explanations for this. Ammonia is a difficult substance to measure accurately in the blood and samples need careful handling [25, 26]. It is therefore possible that the actual levels in our patients were higher than those recorded. In addition, a debate continues as to whether venous or arterial ammonia levels more accurately reflect the situation in vivo [25, 26]. It has also been shown that blood ammonia levels themselves do not always mirror brain ammonia levels and the brain actually concentrates ammonia under hyperammonaemic conditions [25, 27]. A further potential explanation is that there are two types of VE. One produced by a direct toxic effect of VPA or VPA-metabolites on the brain, and the other produced by the mechanism of hyperammonaemia. Two of the patients reported here (patients 4 and 5) had VPA levels exceeding the usual therapeutic range and normal ammonia levels. Although the upper limit of the therapeutic range of VPA does not define a concentration above which toxicity will develop but a serum level beyond which there is a relatively small chance of further seizure improvement, the risk of VPA-related side-effects is greater with higher serum levels [28].
Older people may be at particular risk of direct toxic effects of VPA for a number of reasons including altered pharmacokinetics and comorbidity. Drug and toxicity levels may be affected by age-related changes in gastrointestinal absorption, distribution of drugs due to changes in the fat and water content of the body, liver volume, metabolism and blood flow, or renal excretion rates [7, 29]. Drug-binding to plasma proteins decreases with advancing age. This is particularly relevant for highly protein-bound drugs such as VPA [7]. Older patients with reduced plasma protein binding may have total serum VPA levels within the usual treatment range, but elevated unbound concentrations, putting them at increased risk of side-effects. This may be one reason why the maximum tolerated dose of VPA was less than 1000 mg/day in outpatients with Alzheimer disease not suffering from epilepsy [30]. Moreover, older patients are often receiving concomitant drug therapy for conditions other than epilepsy. This means that there is an increased risk of complex drug-drug interactions in this particular patient group.