Review: Is levetiracetam different from other antiepileptic drugs? Levetiracetam and its cellular mechanism of action in epilepsy revisited

Rainer Surges, Kirill E. Volynski and Matthew C. Walker

Therapeutic Advances in Neurological Disorders 2008 1: 13
DOI: 10.1177/1756285608094212

The online version of this article can be found at:
http://tan.sagepub.com/content/1/1/13

Published by:
SAGE
http://www.sagepublications.com

Additional services and information for Therapeutic Advances in Neurological Disorders can be found at:
Email Alerts: http://tan.sagepub.com/cgi/alerts
Subscriptions: http://tan.sagepub.com/subscriptions
Reprints: http://www.sagepub.com/journalsReprints.nav
Permissions: http://www.sagepub.com/journalsPermissions.nav
Citations: http://tan.sagepub.com/content/1/1/13.refs.html

>> Version of Record - Jul 1, 2008

What is This?
Is levetiracetam different from other antiepileptic drugs? Levetiracetam and its cellular mechanism of action in epilepsy revisited

Rainer Surges, Kirill E. Volynski and Matthew C. Walker

Abstract: Levetiracetam (LEV) is a new antiepileptic drug that is clinically effective in generalized and partial epilepsy syndromes as sole or add-on medication. Nevertheless, its underlying mechanism of action is poorly understood. It has a unique preclinical profile; unlike other antiepileptic drugs (AEDs), it modulates seizure-activity in animal models of chronic epilepsy with no effect in most animal models of acute seizures. Yet it is effective in acute in-vitro ‘seizure’ models. A possible explanation for these dichotomous findings is that LEV has different mechanisms of actions, whether given acutely or chronically and in ‘epileptic’ and control tissue. Here we review the general mechanism of action of AEDs, give an updated and critical overview about the experimental findings of LEV’s cellular targets (in particular the synaptic vesicular protein SV2A) and ask whether LEV represents a new class of AED.

Keywords: levetiracetam, SV2A, antiepileptic drugs, synaptic transmission, epilepsy, ion channels

Introduction
Antiepileptic drug (AED) development has mainly taken place through trial and error. AEDs have been screened in animal models of seizures and epilepsy, often with an incomplete knowledge of their mechanism of action [Walker et al. 2004]. Indeed, the identification of drugs acting at putative ‘antiepileptic’ targets has rarely translated into successful AED therapies, because the drugs are often poorly tolerated or have poor efficacy. Moreover, AEDs that were designed to act at specific targets (e.g., gabapentin, lamotrigine) work via different mechanisms. Consequently, the underlying mechanism of action of an individual drug may only become apparent after its widespread clinical use. However, growing evidence suggests that many of the drugs that we use fall into one or more specific mechanistic groups – drugs that act at sodium channels, calcium channels or the GABAergic system [Walker and Fisher, 2004]. Other putative and potential targets include potassium channels, hyperpolarization-activated cation channels, and glutamate receptors. Here we briefly review the mechanism of action of AEDs and ask whether levetiracetam (LEV) represents a new class of AED.

Main targets for AED

Sodium channels
Sodium channels provide the major target for a number of AEDs including phenytoin, carbamazepine, oxcarbazpine, and lamotrigine. Voltage-gated sodium channels are critical for action potential (AP) generation and propagation [Catterall, 2000a]. The sodium channel exists in three principal conformational states: at hyperpolarized potentials the channel is in the resting closed state; with depolarization the channel opens and permits the conduction of sodium ions; the channel then enters a nonconducted, inactivated state. This inactivation is removed (termed deinactivation) by hyperpolarization. In this manner, depolarization results in a transient inward sodium current that rapidly inactivates. In addition to these three states, there is also a slow inactivated state, which occurs with sustained or repeated depolarizations. This state
is selectively enhanced by the new AED, lacosamide [Errington et al. 2008].

Phenytoin, lamotrigine, oxcarbazepine, and carbamazepine bind to and stabilize the inactivated state of the sodium channel [Kuo, 1998]. This has two effects: a greater proportion of channels are inactive at hyperpolarized membrane potentials, and second there is a delay in deinactivation. The effect on the excitability of neurons is 2-fold. The rate at which an axon can ‘fire’ is critically determined by the rate at which the sodium channels deinactivate. If this time is increased, then the ‘refractory period’ is prolonged, inhibiting sustained repetitive firing [McLean and Macdonald, 1983]. In addition, since these drugs bind to channels in their inactive state, then the greater the number of channels that have entered this state, the greater the drug binding. This results in a ‘use dependent’ phenomenon in which repetitive firing results in greater amounts of the drug bound and so greater inhibition. In addition, these drugs inhibit the persistent sodium current, which mediates long-lasting depolarizations [Lampl et al. 1998]. Other AEDs such as valproate, topiramate, and zonisamide may also have similar effects on sodium channels, but have been less well characterized.

**Calcium channels**

Calcium channels are also putative targets for AEDs, as they regulate not only neuronal excitability but also neurotransmitter release [Catterall, 2000b]. The voltage-gated calcium channels expressed in the brain can be subdivided into four main classes, L-, P/Q-, N-, and T-type channels. L-, P/Q-, and N-type channels are high-voltage activated (HVA) channels that require significant depolarization to open, while the T-type channel is a low-voltage activated (LVA) channel and is opened by relatively small depolarizations.

The L-type channels are mainly expressed postsynaptically. L-type channels are slowly inactivated thereby permitting sustained calcium entry following a depolarization. Calcium entering through L-type calcium channels may play a role in activity-dependent gene expression and synaptic plasticity. Some AEDs (such as carbamazepine) have been proposed to antagonise L-type calcium channels but the relevance of this to their antiepileptic effect is unclear [Ambrosio et al. 1999].

N- and P/Q-type channels are expressed at synaptic boutons where they mediate calcium entry necessary for neurotransmitter release. These channels rapidly inactivate, resulting in brief calcium transients. This calcium entry then triggers exocytosis of presynaptic vesicles. N- and P/Q-type calcium channels can be modulated by G-protein linked receptors such as GABA_B receptors. Inhibition of these channels would be expected to decrease neurotransmitter release. Gabapentin’s and pregabalin’s effect on HVA calcium channels is complex and novel; they both show strong and specific binding for the α2δ auxiliary calcium channel subunit and may modulate P/Q-type calcium channels [Dooley et al. 2007].

T-type calcium channels are activated at relatively hyperpolarized potentials. They open with small depolarization and then rapidly inactivate. They have been proposed to contribute to the generation of physiological rhythms within the thalamus, and have been implicated in the generation of spike-wave discharges associated with absence epilepsy [McCormick and Contreras, 2001]. There is evidence that ethosuximide mediates its effect through binding to and stabilizing the inactivated state of the T-type calcium channel [Gomora et al. 2001]. Other drugs such as zonisamide and valproate have also been suggested to act at this channel [Todorovic and Lingle, 1998; Suzuki et al. 1992].

**GABAergic system**

Gamma amino butyric acid (GABA) is the major inhibitory neurotransmitter in the brain. It is formed and degraded in the GABA shunt. Glutamic acid decarboxylase (GAD) converts glutamate to GABA. Promotion of GABA synthesis has been proposed to contribute to the action of some AEDs including valproate [Löschner, 1989].

GABA is released into the synaptic space where it acts on two receptor types: ionotropic GABA_A and metabotropic GABA_B receptors (a third type, termed GABA_C receptors, is present predominantly in the retina) [Bormann, 2000]. Benzodiazepines act at specific GABA_A receptor subtypes [Mehta and Ticku, 1999], increasing the affinity of GABA_A receptors for GABA, and the probability of receptor opening. Topiramate also potentiates GABA_A receptor currents in a subunit specific manner [Simeone et al. 2006]. Barbiturates are less selective for GABA_A receptor subtypes, and prolong receptor
opening times. Drugs that act at GABA_b receptors have been less useful as AEDs, probably because GABA_b receptors have a complex function acting postsynaptically to decrease neuronal excitability but also presynaptically decreasing GABA release.

GABA is taken up by glial and neuronal GABA transporters, inhibition of which is another AED target (tiagabine) [Rekling et al. 1990]. Inside the cell, GABA is degraded by GABA transaminase to succinic semialdehyde, and inhibition of this enzyme by the AED vigabatrin increases GABAergic transmission [Gale and Iadarola, 1980].

Other targets

Potassium channels
Potassium channels form one of the most diverse groups of ion channels and have a critical role in determining neuronal excitability [Jan and Jan, 1997]. Persistent potassium currents play a crucial part in determining the resting membrane potential of neurons. Voltage-gated potassium channels can influence the resting membrane potential but also repolarize neurons following AP, thereby influencing neurotransmitter release. In addition, the rate of repolarization by potassium channels, affects the ability of a neuron to sustain rapid repetitive firing. Voltage-gated potassium channels in the brain can be subdivided into: channels that rapidly activate and inactivate (A-type channels), channels that open upon depolarization but do not significantly inactivate (delayed rectifier channels) and channels that close upon depolarization but are open at the resting potential (inward rectifying channels). There are other potassium channels that are similar in structure to the voltage-gated potassium channel, but are opened by intracellular calcium (calcium-activated potassium channels that mediate the afterhyperpolarization) or by cyclic nucleotides (mainly present in the retina where they mediate photoreceptor responses). There are also specific potassium channels that are inactivated by acetylcholine – termed M-type channels. Although, modulation of potassium channels would seem to be an ideal target for AEDs, most drugs have no or poorly characterized effects on potassium channels. However, phenytoin blocks the delayed rectifier potassium channels in neuroblastoma cells and retigabine, a putative AED, has as its main mode of action potentiation of potassium M-type channels [Wuttke et al. 2005; Tatulian et al. 2001; Nobile and Lagostena, 1998].

HCN channels
HCN channels are permeable to both potassium and sodium and mediate a current termed the H-current. These channels are activated at hyperpolarised potentials and deactivated at depolarized potentials. H-currents depolarize neurons from the resting membrane potential and have an important role in potentiating and maintaining oscillations [Robinson and Siegelbaum, 2003]. They may play a part in terminating thalamic oscillations and the generation of spike-wave discharges of absence epilepsy. The H-current is also highly expressed in dendrites where it shunts excitatory inputs. Lamotrigine has been shown to enhance the H-current in dendrites [Poolos et al. 2002]. Likewise, gabapentin has been demonstrated to increase the H-current in pyramidal neurons [Surges et al. 2003]. This may have two potentially antiepileptic effects: in the hippocampus it would inhibit excitatory transmission to the soma, explaining the efficacy of lamotrigine and gabapentin in partial epilepsy. In the thalamus, it may inhibit or terminate spike-wave discharges and, therefore, could explain the efficacy of lamotrigine against absence seizures.

Glutamate and glutamate receptors
Glutamate is the major excitatory transmitter in the central nervous system and acts at distinct receptor types: N-methyl-D-aspartate (NMDA), non-NMDA [consisting of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainic acid (KA) sensitive receptors] and metabotropic glutamate receptors. Inhibition of these receptors would seem to be an ideal target for AEDs, but such compounds have been associated with unacceptable side-effects. NMDA receptors influence memory, cognition, and learning and NMDA receptor antagonists have had unacceptable side effects in clinical use. Felbamate and remacemide, however, may modulate NMDA receptor-mediated transmission [Subramaniam et al. 1996; White et al. 1995].

Topiramate at high concentrations acts at AMPA/kainate receptors; whether this is responsible for its antiepileptic effect or dose-related side effects is unknown [Angehagen et al. 2004]. Low doses of phenobarbitone have been shown to block
AMPA receptors in the cerebral cortex [Sawada and Yamamoto, 1985], but the significance of this finding and its overall contribution towards the antiepileptic effects of phenobarbitone remains to be established. There are other drugs in clinical trials such as talampanel that are AMPA receptor antagonists.

**Levetiracetam**

LEV is a water soluble pyrrolidone derivative ((S)-α-ethyl-2-oxo-pyrrolidine acetamide), whose chemical structure differs from other AEDs. Since its approval for clinical use in 2002, LEV has become a widely used AED that is effective in partial and generalized epilepsy syndromes as sole or add-on medication [De Smedt et al. 2007]. Usual antiepileptic plasma concentrations range from trough levels between 35 and 100 µM (5.95–17 µg/ml) to peak levels between 90 and 250 µM (15.3–42.5 µg/ml) [Rigo et al. 2002; Patsalos, 2000]. Importantly, serum levels of LEV are very similar to corresponding LEV levels found in the brain tissue of individual patients [Rambeck et al. 2006]. Unlike other AEDs, LEV is probably not a substrate for multidrug transporters [Potschka et al. 2004].

Except for rare instances of the treatment of acute seizures, AED therapy involves a regular daily, therefore chronic, intake of medication. Therefore, acute in-vitro and in-vivo experimental paradigms do not necessarily reflect the clinical use of an AED. Moreover, there are various epilepsy-associated modifications of brain physiology, and therefore models of acute seizures differ from models of chronic epilepsy. Intriguingly, LEV modulates seizure activity in animal models of chronic epilepsy (kindling models, pilocarpine model, genetic absence epilepsy rats from Strasbourg GAERS) with no effect in most models of acute seizures [Glien et al. 2002; Klitgaard et al. 1998; Lőscher and Hönack, 1993]. This is consistent with the experimental observations that LEV only affects GABA<sub>A</sub> receptors from epileptic tissue or under conditions that occur during epilepsy, whereas it has no effect on GABA<sub>A</sub> receptors from controls [Palma et al. 2007; Rigo et al. 2002]. Taken together, these data suggest that LEV may preferentially work with chronic application or under chronic epilepsy-associated conditions. However, most of the experiments to investigate LEV’s cellular mechanism of action have been performed by acute application, reporting its acute cellular effects. A further consideration is that LEV is now being proposed as an acute treatment for seizures. An intravenous formulation is available [Ramael et al. 2006] that has already been shown to terminate status epilepticus after acute intravenous application [Knake et al. 2008]. Interestingly, LEV is effective in one model of acute epilepsy (6 Hz psychomotor seizure model) [Shannon et al. 2005; Barton et al. 2001] with the maximal effect occurring 1 h after injection [Barton et al. 2001]. These findings suggest that LEV can have a rapid-onset effect in some acute seizure models. One possible explanation for these dichotomous findings in animal models of acute and chronic epilepsy is that LEV may have different mechanisms of action whether given acutely or chronically and in epileptiform and control tissue (see subsequently).

**Action of LEV on voltage-gated ion channels and regulation of intracellular ions**

Neuronal excitability and firing behavior are crucially shaped by voltage-gated ion channels. Acute application of LEV at a relatively low concentration (10 µM) did not alter neuronal properties such as membrane potential, input resistance, AP amplitude, AP duration, or fast and slow afterhyperpolarization of CA3 pyramidal cells [Birnstiel et al. 1997]. However, at higher concentrations (similar to those used in clinical practice) there is substantial experimental evidence that acutely applied LEV (and prolonged application for up to 1 h) modulates cellular targets that are important for neuronal excitability and synaptic transmission (cf. Tables 1 and 2).

**Voltage-gated ion channels**

HVA Ca<sup>2+</sup> currents in different cell preparations (acutely isolated striatal, neocortical, and hippocampal CA1 neurons, CA1 pyramidal neurons in slices) were inhibited by an average of 18–40% when LEV was acutely applied at different concentrations (1–300 µM) [Costa et al. 2006; Pisani et al. 2004; Lukyanetz et al. 2002; Niespodziany et al. 2001]. Pharmacological separation of different HVA Ca<sup>2+</sup> channel subtypes revealed that mainly N-type, and to a lesser extent P/Q-type calcium channels were affected [Costa et al. 2006; Pisani et al. 2004; Lukyanetz et al. 2002]. Changes in steady-state activation or inactivation properties were not observed [Lukyanetz et al. 2002]. In contrast, LEV did not modulate amplitudes, steady-state activation/inactivation properties or kinetics of T-type Ca<sup>2+</sup> currents...
A reduction of N-type and P/Q-type calcium currents can lead to a decrease in presynaptic Ca\(^{2+}\)-dependent processes involved in neurotransmitter release. These channels have been implicated in both ictogenesis and epileptogenesis.

Delayed rectifier K\(^{+}\) currents were decreased by about 30% when LEV was acutely applied to CA1 neurons, whereas A-type K\(^{+}\) currents were unaffected [Madeja et al. 2003]. The reduction of delayed rectifier K\(^{+}\) currents led to a reduction in repetitive AP generation and a slight prolongation of AP duration. The relevance of this to its antiepileptic effect remains unknown, but may decrease neuronal firing.

Unlike some classical AEDs, acute and prolonged application (for up to 1 h) of LEV at different concentrations (ranging from 1 μM–1 mM) in different cell preparations (acutely isolated striatal and hippocampal CA1 neurons, cultured neocortical neurons) had no effect on either the amplitudes of fast Na\(^{+}\) currents [Costa et al. 2006; Madeja et al. 2003; Zona et al. 2001] or on steady-state activation and inactivation properties or current kinetics [Zona et al. 2001]. Likewise, persistent Na\(^{+}\) currents were not affected [Niespodziany et al. 2004]. However, activity-dependent inhibition (use-dependence) of Na\(^{+}\) currents has, to our knowledge, not been investigated, but given the above findings it is unlikely that LEV would have an effect.

**Table 1.** LEV effects on voltage-gated ion currents.

| Effect                  | Concentration | Application time | Tissue                      | Reference                  |
|-------------------------|---------------|------------------|-----------------------------|----------------------------|
| **Na\(^{+}\)** currents |               |                  |                             |                            |
| Fast Na\(^{+}\) currents|               |                  |                             |                            |
| No effect               | 10 μM to 1 mM | Acute up to 10 min | Cultured neocortical neurons | Zona et al. 2001           |
| No effect               | 100 μM       | Acute            | Acutely isolated CA1 neurons | Madeja et al. 2003         |
| No effect               | 1–500 μM     | Probably acute   | Acutely isolated striatal neurons | Costa et al. 2006         |
| No effect               | 10–100 μM    | Acute for up to 1 h | CA1 neurons in slices | Niespodziany et al. 2004 |
| Persistent Na\(^{+}\) currents|          |                  |                             |                            |
| No effect               |               |                  |                             |                            |
| **Ca\(^{2+}\)** currents |               |                  |                             |                            |
| HVA                     |               |                  |                             |                            |
| Inhibition by ~30%      | 32 μM         | Acute up to 30 min | CA1 pyramidal neurons in slices | Niespodziany et al. 2001 |
| Inhibition by ~18% via N-type Ca\(^{2+}\) channel blockade | 200 μM | Acute | Acutely isolated hippocampal CA1 neurons | Lukyanetz et al. 2002 |
| Inhibition by 35%, mainly via blockade of N-type and to a lower extent of P/Q-type Ca\(^{2+}\) channels | 100 μM | Acute | Acutely isolated neocortical neurons | Pisani et al. 2004 |
| Inhibition by 40% (30% N-type, 10% P-type) | Half-maximal inhibition at 22 μM | Probably acute | Acutely isolated striatal neurons | Costa et al. 2006 |
| LVA                     |               |                  |                             |                            |
| No effect on T-type Ca\(^{2+}\) currents | 32–100 μM | Acute for up to 40 min | CA1 pyramidal neurons in slices | Zona et al. 2001 |
| **K\(^{+}\)** currents |               |                  |                             |                            |
| Delayed rectifier K\(^{+}\) current | Inhibition by ~30% | 100 μM | Acute | Acutely isolated CA1 neurons | Madeja et al. 2003 |
| A-type K\(^{+}\) current | No effect | 100 μM | Acute | Acutely isolated CA1 neurons | Madeja et al. 2003 |

Intraneuronal Ca\(^{2+}\) stores and Cl\(^{-}\)/HCO\(_{3}^{-}\) exchanger

Intriguingly, other cellular targets for LEV that are involved in the regulation of neuronal excitability have recently been identified, including intraneuronal calcium stores. These play an important role in the regulation of neuronal excitability, neurotransmission and synaptic plasticity as well as disease-related processes such as epileptogenesis and seizure-like activity [Bardo et al. 2006; Pal et al. 2001]. In most neurons Ca\(^{2+}\)-release from these stores is mainly
regulated by inositol (1,4,5)-triphosphate (IP3) and ryanodine receptors with varying relative contributions of each. Activation of ryanodine receptors by caffeine in mixed hippocampal cell cultures led to a transient increase of intracellular Ca\(^{2+}\) as well as to spontaneous bursts in hippocampal slices. These Ca\(^{2+}\) transients were reduced by almost 50% after 5 min incubation with LEV and occurrence of spontaneous bursts in slices was delayed by LEV [Angehagen et al. 2003]. Furthermore, Gq-protein coupled IP3-dependent Ca\(^{2+}\) release in rat PC12 pheochromocytoma cells was inhibited by about 25–50% after 5 min incubation with LEV [Cataldi et al. 2005]. Thus, inhibition of Ca\(^{2+}\) release from intraneuronal Ca\(^{2+}\) stores induced by LEV may contribute to its antiepileptic effects.

The function of many proteins is pH dependent. Changes in intraneuronal pH modulate neuronal activity and intraneuronal acidification reduces seizure-like activity in in-vitro preparations [Bonnet et al. 2003]. In hippocampal slices acutely applied LEV for up to 20 min acidified the internal pH of CA3 neurons [Leniger et al. 2004]. Moreover, LEV administration decreased the frequency of spontaneous AP and bursts induced by 4-aminopyridine. Both effects were reversible upon washout and the latter could be reversed by application of a membrane-permeable base. Further analysis revealed that the acidification in the presence of LEV was probably linked to the blockade of the Na\(^{+}\)-dependent Cl\(^{-}\)/HCO\(_3\)^{-} exchanger. This exchanger participates in the extrusion of intracellular acid, thus a LEV-induced blockade was proposed to acidify the intracellular milieu, thereby reducing seizure-like activity [Leniger et al. 2004].

### Table 2. LEV effects on ligand-gated ion currents and other targets.

| Effect                      | Concentration | Application time | Tissue                              | Reference                      |
|-----------------------------|---------------|------------------|-------------------------------------|--------------------------------|
| AMPA receptors              | Inhibition by 10–25% | 200 µM           | Acute                              | Carunchio et al. 2007         |
| GABAA receptors             | Complete reversal of zinc-induced inhibition | 30 µM           | Acute                              | Rigo et al. 2002              |
| Glycine receptors           | Complete reversal of zinc-induced inhibition | Half-maximal effect at 0.04 µM | Acute                              | Rigo et al. 2002              |
| Ca\(^{2+}\) stores          | Ryanodine-regulated Ca\(^{2+}\) release | Inhibition by ~50% | After 5 min                          | Angehagen et al. 2003         |
|                             | IP3-regulated Ca\(^{2+}\) release | Inhibition by 25–50% | After 5 min                          | Cataldi et al. 2005           |
| Cl\(^{-}\)/HCO\(_3\)^{-} exchanger | Inhibition | 10–50 µM         | Up to 20 min                        | Leniger et al. 2004           |

### Action of LEV on synaptic transmission

AEDs can act at pre- or post-synaptic sites to modulate synaptic transmission. Evoked presynaptic release of neurotransmitters is triggered by an AP-induced calcium influx via P/Q-, N-, and R-type calcium channels. We have already described the effect of LEV on presynaptic calcium channels. In the following paragraphs we will further discuss LEV action on synaptic transmission including its interaction with presynaptic vesicular proteins and modulation of post-synaptic ligand-gated receptors.

### Synaptic vesicle binding site for LEV

Lynch et al. (2004) showed that LEV specifically binds to the synaptic vesicle protein SV2A, whereas it does not bind to its two isoforms SV2B or SV2C. Moreover it was demonstrated that SV2A binding affinity of different LEV derivatives positively correlated with their antiepileptic potency in different animal models of epilepsy [Kaminski et al. 2008; Lynch 2004]. These findings strongly suggest that LEV binding to SV2A is involved in its antiepileptic effect. Indeed, SV2A knock-out (KO) mice strains display a severe seizure
phenotype after the first postnatal week [Crowder et al. 1999; Janz et al. 1999] indicating that SV2A may normally regulate signaling cascades involved in seizure generation. Unfortunately no data on the actual CSF levels of different LEV derivatives were provided in the above correlation studies. Thus, it is still possible that the potency of different LEV derivatives in seizure-prevention was related to different CSF levels rather than to different binding affinities to SV2A. It is also unclear how these LEV derivatives act on voltage- or ligand-gated ion channels involved in synaptic transmission.

SV2 is a major vesicular protein that contains 12 putative transmembrane regions and resembles membrane transporters. [Janz et al. 1999]. Unfortunately to date, there are no available reports on the specific binding site for LEV on the SV2A molecule. Also the functions of SV2 proteins in synaptic transmission are still debated. Electrophysiological recordings in cultured autaptic hippocampal neurons (which form synapses with themselves) from single and double SV2A/SV2B KO mice suggest several, somewhat disparate, possible mechanisms by which SV2 may regulate vesicular exocytosis. At high extracellular calcium concentrations (i.e., when the initial release probability is high) neurons from the SV2A/SV2B double KO exhibited no change in the initial probability of release but revealed a sustained increase in the AP-evoked synaptic transmission during trains of AP [Janz et al. 1999]. Importantly the increase could be partially reversed by loading presynaptic terminals with slow calcium buffer EGTA. These results prompted two alternative hypotheses: (i) the function of SV2s is to regulate presynaptic calcium levels during repetitive activity or (ii) SV2s function as targets for residual calcium in regulating vesicular exocytosis. In contrast, when synaptic transmission was assessed at physiological extracellular calcium concentrations, loss of SV2A, and SV2B led to reduced initial release probability but had no effect on steady-state responses during the trains of AP [Custer, 2006]. These results suggest that SV2A may regulate priming of docked synaptic vesicles, a process that makes them ready for exocytosis, and thus selectively enhancing low frequency neurotransmission.

Overall, although SV2A was identified as a specific binding site for LEV, a direct demonstration of SV2A mediating the antiepileptic effect of LEV via a change in synaptic transmission is still lacking.

Synaptic transmission in-vivo

In-vivo studies are an important tool to judge the overall efficacy of AEDs in native environment of preserved neuronal circuitry. However, very often it is not possible to precisely control the AED concentrations during an in-vivo experiment. This in turn makes difficult dissecting specific AED actions on different signaling pathways. The effects of acute and chronic LEV application on neurochemical parameters in the mouse brain were examined after single or repeated intraperitoneal LEV injections [Sills et al. 1997]. No effect of LEV on whole brain preparations was detected either on the overall GABA and glutamate concentrations or on the overall activities of GABA transaminase and GAD. In contrast, when studied in-vivo on a regional basis, acute LEV application had different effects within different brain regions with the most pronounced (and opposite) effects on GABA turnover in the cortex and striatum [Lösch et al. 1996]. The implications for this finding are uncertain but suggest that LEV may operate through different mechanisms in different brain areas.

Acute systemic application of LEV had no effect on evoked population spikes in the hippocampal CA3 region recorded in-vivo, but prevented the increase of the population-spikes induced by local application of bicuculline [Margineau and Wülfert, 1995]. Since the paired-pulse ratio in the presence or absence of bicuculline was not altered by LEV, LEV probably has little effect at presynaptic sites when applied acutely. Furthermore, acute LEV prevented the bicuculline-induced increase in population-spikes probably independent of a GABAAergic pathway [Margineau and Wülfert, 1995]. In addition, in in-vivo experiments, acute systemic application of antiepileptic LEV doses also failed to alter evoked field potentials and GABAAergic (GABA_A and GABA_B receptor) mechanisms in the hippocampal dentate gyrus [Margineau and Klitgaard, 2003].

Synaptic transmission in-vitro

As stated in the previous paragraph, there is no electrophysiological evidence for a direct effect of acutely applied LEV on the pre- or postsynaptic site of naïve GABAAergic synapses. Further, there is no direct LEV effect on naïve GABA_A receptor mediated inhibitory currents in
neuronal cell cultures, or in hippocampal and hypothalamic slices [Poulain and Margineanu, 2002; Rigo et al. 2002; Birnstiel et al. 1997]. Intriguingly, however, there have been two studies describing an effect of LEV on GABA_A receptors that have undergone modifications associated with epilepsy. First, in hippocampi from epileptic brains GABA_A receptor function can be impaired via allosteric inhibition by zinc, thereby reducing the overall inhibitory effect of GABA [Coulter 2000]. This zinc-induced inhibition of GABA_A receptors was fully reversed by acute application of LEV in cultured hippocampal neurons, whereas GABA_A-induced currents in controls were unchanged [Rigo et al., 2002]. Second, Palma et al. (2007) prepared neuronal membranes from human tissue removed during brain surgery. They then micro-transplanted the membrane preparations into frog oocytes and investigated evoked GABA_A receptor currents. The GABA_A receptor currents from epileptic hippocampi were substantially impaired upon repeated GABA application without changes in decay time. This run-down was substantially reduced by pre-incubation with LEV for 3 h, suggesting stabilization of GABA_A receptor currents during repeated activation. The stabilizing effect was also present with testing on GABA_A receptor currents in neocortical pyramidal neurons in slices from temporal lobe epilepsy patients. This particular effect probably involved a protein kinase C dependent pathway, needed more than 1 h to develop and slowly diminished upon withdrawal of LEV.

LEV may also reduce glutamatergic transmission. Whereas acutely applied LEV at a low concentration (10 μM) had no appreciable effect on evoked excitatory postsynaptic potentials (EPSPs) in hippocampal slices [Birnstiel et al. 1997], acutely applied LEV at concentrations within the usual clinical range (100–300 μM) decreased evoked EPSPs by about 20% in striatal slices [Costa et al. 2006]. This effect was probably not due to an inhibition of presynaptic N- and P/Q-type Ca^{2+} channels, since the paired-pulse ratio (an indicator of a presynaptic site of action) was unaltered. Interestingly, LEV acutely applied to cultured cortical neurons reversibly reduced AMPA-receptor currents by ~25% [Carunchio et al. 2007]. However, Yang et al. (2007) did not observe any change in evoked field potentials in the CA1 region of hippocampal slices during acute LEV application. In contrast, 3 h pre-incubation of the slices with 100 μM LEV resulted in a relative decrease in the amplitude of late field potentials during an 80 Hz train. Thus, LEV exhibited a use-dependent decrease in neurotransmitter release. The same group also showed that, using the FM dye technique, the synaptic exocytosis rate at 1 Hz stimulation was diminished after pre-incubation with LEV, suggesting a presynaptic site of action. Unfortunately, these imaging experiments did not distinguish between LEV’s action on glutamatergic or GABAergic terminals, and thus the effect of this mechanism on network behavior remains unclear.

**Action of LEV on in-vitro models of epilepsy**

So far, we have reviewed the cellular targets of LEV that were mostly identified in-vitro under ‘physiological’ conditions. One way to judge the anticipated antiepileptic efficacy of a drug is to apply it to in-vitro models of acute seizures. Surprisingly, in contrast to most in-vitro animal models of acute seizures (apart from the 6 Hz psychomotor seizure model, Barton et al. 2001), LEV had significant effects in in-vitro seizure models. Spontaneous NMDA-induced epileptiform bursts in hippocampal slices were substantially reduced during acute application of 10 μM LEV (with a maximum effect 20–30 min after drug application) [Birnstiel et al. 1997]. Similarly acute bath application of LEV at concentrations between 10–500 μM led to consistent reduction of seizure-like activity induced by bicuculline in both human neocortical slices from epileptic patients and in rat hippocampal slices [Gorji et al. 2002; Birnstiel et al. 1997]. The underlying mechanism is likely unrelated to a direct effect of LEV on GABA_A receptors and remains unclear. LEV has also been tested in a different model in which reduction of extracellular magnesium, application of bicuculline and 4-aminopyridine led to spontaneous seizure-like activity characterized by paroxysmal depolarization shifts in pyramidal neurons of neocortical brain slices [Pisani et al. 2004]. In this model, acute application of LEV for 10–20 min reversibly decreased the duration of the paroxysmal depolarization shift as well as the relative increase in calcium with a half-maximal inhibition at concentrations between 140 and 180 μM LEV. In hippocampal slices, bath application of caffeine-induced spontaneous bursts whose occurrence was considerably delayed after 5 min incubation with LEV [Angehagen et al. 2003], suggesting an inhibitory effect of LEV on Ca^{2+} release from intracellular stores. Moreover, LEV administration decreased the frequency of spontaneous
APs and bursts induced by 4-aminopyridine [Leniger et al. 2004] that could be linked to a LEV-induced intracellular acidification. Surprisingly and unlike other AEDs with high extracellular K⁺ and low Ca²⁺ (both of which increase neuronal excitability), 32 μM LEV (application for up to 80 min) selectively impaired the number of evoked epileptiform and hypersynchronous field potentials in CA3 area of hippocampal slices without altering the number of APs recorded intracellularly [Nespodziany et al. 2003]. These results suggest an uncoupling of interneuronal synchrony; however, its underlying mechanism remains to be elucidated.

Taken together, most of the observed ‘anti epileptiform’ effects of LEV in-vitro can be explained by the sum of the known mechanisms of action, although it is still difficult to dissect out which are most important. This raises the question of why there is an ‘anti epileptic’ LEV effect in in-vitro, but not in most of the in-vivo models of acute seizures. This may be due to obtaining a sufficiently high and persistent LEV concentration in brain slices or in cell cultures as compared to in-vivo models with acute systemic LEV application. In the latter, there may be complex concentration profiles in different brain areas with an insufficient total increase or local increase in the seizure generating areas of LEV. Alternatively, specific models of acute seizures in-vivo models may select for AEDs with distinct mechanisms of actions (i.e., generation and propagation of ictal activity in a specific model may be critically dependent on particular pathways and cellular targets). If this is true, why is LEV effective in chronic models of epilepsy? Epileptic tissues undergo various alterations in voltage- and ligand-gated ion channels [Walker et al. 2007]. As already described beforehand, e.g., GABA_A receptors from ‘epileptic’ tissue or under conditions occurring in chronic epilepsy (e.g., zinc-induced allosteric inhibition) were modulated by LEV. Thus, ‘epileptic’ tissue could be more sensitive to LEV than ‘nonepileptic’ tissue and so require lower LEV concentrations. At the same time, drug resistance and tolerance, that is a reduction in response to a drug after repeated applications, can occur during chronic treatment or long-standing epilepsy that might be related to target-specific compensatory mechanisms or disease-specific modifications. Indeed, development of drug tolerance and resistance in animal models probably depends on the specific epilepsy models used and can be highly selective for specific AEDs (e.g., LEV is effective in phenytoin-resistant amygdala-kindled rats [Lösch et al. 2000]).

Summary and conclusions
Unlike other AEDs, LEV exerts antiepileptic effects predominantly in animal models of chronic epilepsy with no effect in most common animal models of acute epilepsy. This is consistent with the observation that LEV only affected GABA_A receptors from ‘epileptic’ tissue or under conditions that occur during epilepsy, whereas naïve GABA_A receptors were not modulated. However, LEV is effective in in-vitro models of acute epilepsy and also in one animal model of acute epilepsy. Moreover, an increasing number of naïve cellular targets for LEV have been identified to date whose modulation has potential antiepileptic effects. From these studies, it emerges that LEV may have preferential sites and mechanisms of action, depending on the speed of administration and the condition of the tissue. Acutely applied LEV in clinically relevant concentrations may preferentially modulate neuronal activity by inhibition of intracellular Ca²⁺ increase (via blockade of N- and P/Q-type Ca²⁺ channels and Ca²⁺ release from intraneuronal stores), delayed rectifier K⁺ currents, Cl⁻/HCO₃⁻ exchanger and AMPA-receptors (cf. Tables 1 and 2). In contrast and unlike other AEDs, chronically applied LEV may predominantly act via binding to SV2A, probably leading to decreased transmitter release, which, together with the modulation of ion channels and other targets, potently prevents epileptic activity. In tissue of epileptic patients, the disease-associated modifications may make neuronal circuits more sensitive to LEV and new targets may emerge, such as altered GABA_A receptors, thereby strengthening inhibitory neurotransmission.

In conclusion, although LEV shares some targets (such as delayed rectifier channels and N- and P/Q-type calcium channels) with other AEDs, LEV exhibits unique mechanisms of action and a novel and unique binding site.

Acknowledgment
This work was supported by the Deutsche Forschungsgemeinschaft (RS) and CRDC (Clinical Research and Development Committee of Royal Free and University
College Medical School) at the University College London (KEV).

Conflict of interest statement
None declared.

References
Ambrosio, A.F., Silva, A.P., Malva, J.O., Soares-da-Silva, P., Carvalho, A.P. and Carvalho, C.M. (1999) Carbamazepine inhibits L-type Ca\(^{2+}\) channels in cultured rat hippocampal neurons stimulated with glutamate receptor agonists. *Neuropharmacology* 38: 1349–1359.

Angehagen, M., Margineanu, D.G., Ben-Menachem, E., Rönnbäck, L., Hansson, E. and Klinggaard, H. (2003) Levetiracetam reduces caffeine-induced Ca\(^{2+}\) transients and epileptiform potentials in hippocampal neurons. *Neuroreport* 14: 471–475.

Angehagen, M., Ben-Menachem, E., Shank, R., Rönnbäck, L. and Hansson, E. (2004) Topiramate modulation of kainate-induced calcium currents is inversely related to channel phosphorylation level. *J Neurochem* 88: 320–325.

Bardo, S., Cavazzini, M.G. and Empgate, N. (2006) The role of the endoplasmic reticulum Ca\(^{2+}\) store in the plasticity of central neurons. *Trends Pharmacol Sci* 27: 78–84.

Barton, M.E., Klein, B.D., Wolf, H.H. and White, H.S. (2001) Pharmacological characterization of the 6 Hz psychomotor seizure model of partial epilepsy. *Epilepsy Res* 47: 217–227.

Bimstiel, S., Wülffert, E. and Beck, S.G. (1997) Levetiracetam (ucb LO59) affects in vitro models of epilepsy in CA3 pyramidal neurons without altering normal synaptic transmission. *Naunyn Schmiedebergs Arch Pharmacol* 356: 611–618.

Bonnet, U., Bingmann, D. and Wiemann, M. (2000) Intracellular pH modulates spontaneous and epileptiform bioelectric activity of hippocampal CA3-neurones. *Eur Neuropsychopharmacol* 10: 97–103.

Bormann, J. (2000) The ‘ABC’ of GABA receptors. *Trends Pharmacol Sci* 21: 16–19.

Carunchio, I., Pieri, M., Ciotti, M.T., Albo, F. and Zona, C. (2007) Modulation of AMPA receptors in cultured cortical neurons induced by the antiepileptic drug levetiracetam. *Epilepsia* 48: 654–662.

Cataldi, M., Lariccia, V., Secondo, A. di Renzo, G. and Annunziato, L. (2005) The antiepileptic drug levetiracetam decreases the inositol 1,4,5-trisphosphate-dependent [Ca\(^{2+}\)]I increase induced by ATP and bradykinin in PC12 cells. *J Pharmacol Exp Ther* 313: 720–730.

Catterall, W.A. (2000b) Structure and regulation of voltage-gated Ca\(^{2+}\) channels. *Annu Rev Cell Dev Biol* 16: 521–555.

Costa, C., Martella, G., Picconi, B., Prosperetti, C., Pisani, A., Di Filippo, M. et al. (2006) Multiple mechanisms underlying the neuroprotective effects of antiepileptic drugs against in vitro ischemia. *Stroke* 37: 1319–1326.

Coulter, D.A. (2000) Mossy fiber zinc and temporal lobe epilepsy: pathological association with altered ‘epileptic’ gamma-aminobutyric acid A receptors in dentate granule cells. *Epilepsia* 41: S96–S99.

Crowder, K.M., Gunther, J.M., Jones, T.A., Hale, B.D., Zhang, H.Z., Peterson, M.R. et al. (1999) Abnormal neurotransmission in mice lacking synaptic vesicle protein 2A (SV2A). *Proc Natl Acad Sci USA* 96: 15268–15273.

Custer, K.L., Austin, N.S., Sullivan, J.M. and Bajjalieh, S.M. (2006) Synaptic vesicle protein 2 enhances release probability at quiescent synapses. *J Neurosci* 26: 1303–1313.

De Smedt, T., Raedt, R., Vonck, K. and Boon, P. (2007) Levetiracetam: part II, the clinical profile of a novel anticonvulsant drug. *GNS Drug Rev* 13: 57–78.

Dooley, D.J., Taylor, C.P., Donevan, S. and Feltner, D. (2007) Ca\(^{2+}\) channel alpha2delta ligands: novel modulators of neurotransmission. *Trends Pharmacol Sci* 28: 75–82.

Errington, A.C., Stöhr, T., Heers, C. and Lees, G. (2008) The investigational anticonvulsant lacosamide selectively enhances slow inactivation of voltage-gated sodium channels. *Mol Pharmacol* 73(1): 157–169.

Gale, K. and Iadarola, M.J. (1980) Seizure protection and increased nerve-terminal GABA: delayed effects of GABAA transaminase inhibition. *Science* 208: 288–291.

Glen, M., Brandt, C., Potschka, H. and Löschner, W. (2002) Effects of the novel antiepileptic drug levetiracetam on spontaneous recurrent seizures in the rat pilocarpine model of temporal lobe epilepsy. *Epilepsia* 43: 350–357.

Gomora, J.C., Daud, A.N., Weiergraber, M. and Perez-Reyes, E. (2001) Block of cloned human T-type calcium channels by succinimide antiepileptic drugs. *Mol Pharmacol* 60: 1121–1132.

Gorji, A., Höhling, J.M., Madeja, M., Straub, H., Köhling, R., Tuxhorn, I. et al. (2002) Effect of levetiracetam on epileptiform discharges in human neocortical slices. *Epilepsia* 43: 1480–1487.

Jan, L.Y. and Jan, Y.N. (1997) Cloned potassium channels from eukaryotes and prokaryotes. *Annu Rev Neurosci* 20: 91–123.

Janz, R., Goda, Y., Geppert, M., Missler, M. and Südhof, T.C. (1999) SV2A and SV2B function as molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron* 26: 13–25.
Kaminski, R.M., Matagne, A., Leclercq, K., Gillard, M., Michel, P., Kenda, B. et al. (2008) SV2A protein is a broad-spectrum anticonvulsant target: functional correlation between protein binding and seizure protection in models of both partial and generalized epilepsy. *Neuropharmacology* 54: 715–720.

Klitgaard, H., Matagne, A., Gobert, J. and Wulfert, E. (1998) Evidence for a unique profile of levetiracetam in rodent models of seizures and epilepsy. *Eur J Pharmacol* 353: 191–206.

Knake, S., Gruener, J., Hattemer, K., Klein, K.M., Bauer, S., Oertel, W.H. et al. (2008) Intravenous levetiracetam in the treatment of benzodiazepine refractory status epilepticus. *J Neurol Neurosurg Psychiatry* 79: 588–589.

Kuo, C.C. (1998) A common anticonvulsant binding site for phenytoin, carbamazepine, and lamotrigine in neuronal Na⁺ channels. *Mol Pharmacol* 54: 712–721.

Lampi, I., Schwindt, P. and Crill, W. (1998) Reduction of cortical pyramidal neuron excitability by the action of phenytoin on persistent Na⁺ current. *J Pharmacol Exp Ther* 284: 228–237.

Leniger, T., Thöne, J., Bonnet, U., Hufnagel, A., Bingmann, D. and Wiemann, M. (2004) Levetiracetam inhibits Na⁺-dependent Cl⁻/HCO₃⁻ exchange of adult hippocampal CA3 neurons from guinea-pigs. *Br J Pharmacol* 142: 1073–1080.

Lösch, W. (1989) Valproate enhances GABA turnover in the substantia nigra. *Brain Res* 501: 198–203.

Lösch, W. and Hönack, D. (1993) Profile of ucb L059, a novel anticonvulsant drug, in models of partial and generalized epilepsy in mice and rats. *Eur J Pharmacol* 232: 147–158.

Lösch, W., Hönack, D. and Bloms-Funke, P. (1996) The novel antiepileptic drug levetiracetam (ucb L059) induces alterations in GABA metabolism and turnover in discrete areas of rat brain and reduces neuronal activity in substantia nigra pars reticulata. *Brain Res* 735: 208–216.

Lösch, W. and Hönack, D. (2000) Development of tolerance during chronic treatment of kindled rats with the novel antiepileptic drug levetiracetam. *Epilepsia* 41: 1499–1506.

Lösch, W., Reissmüller, E. and Ebert, U. (2000) Anticonvulsant efficacy of gabapentin and levetiracetam in phenytoin-resistant kindled rats. *Epilepsy Res* 40: 63–77.

Lukyanetz, E.A., Shkryl, V.M. and Kostyuk, P.G. (2002) Selective blockade of N-type calcium channels by levetiracetam. *Epilepsia* 43: 9–18.

Lynch, B.A., Lambeng, N., Nocka, K., Kennes-Hamme, P., Bajalieh, S.M., Matagne, A. et al. (2004) The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam. *Proc Natl Acad Sci USA* 101: 9861–9866.

Madeja, M., Margineanu, D.G., Gorji, A., Sipe, E., Boerigter, P., Klitgaard, H. et al. (2003) Reduction of voltage-operated potassium currents by levetiracetam: a novel antiepileptic mechanism of action? *Neuropharmacology* 45: 661–671.

Margineanu, D.G. and Wulfert, E. (1998) ucb L059, a novel anticonvulsant, reduces bicuculline-induced hyperexcitability in rat hippocampal CA3 in vivo. *Eur J Pharmacol* 286: 321–325.

Margineanu, D.G. and Klitgaard, H. (2003) Levetiracetam has no significant gamma-aminobutyric acid-related effect on paired-pulse interaction in the dentate gyrus of rats. *Eur J Pharmacol* 466: 255–261.

McCormick, D.A. and Contreras, D. (2001) On the cellular and network basis of epileptic seizures. *Annu Rev Physiol* 63: 815–846.

McLean, M.J. and Macdonald, R.L. (1983) Multiple actions of phenytoin on mouse spinal cord neurons in cell culture. *J Pharmacol Exp Ther* 227: 779–789.

Mehta, A.K. and Ticku, M.K. (1999) An update on GABAA receptors. *Brain Res Brain Res Rev* 29: 196–217.

Niespodziany, I., Klitgaard, H. and Margineanu, D.G. (2001) Levetiracetam inhibits the high-voltage-activated Ca(2+) current in pyramidal neurones of rat hippocampal slices. *Neurosci Lett* 306: 5–8.

Niespodziany, I., Klitgaard, H. and Margineanu, D.G. (2003) Desynchronizing effect of levetiracetam on epileptiform responses in rat hippocampal slices. *Neuroreport* 14: 1273–1276.

Niespodziany, I., Klitgaard, H. and Margineanu, D.G. (2004) Is the persistent sodium current a specific target of anti-absence drugs? *Neuroreport* 15: 1049–1052.

Nobile, M. and Lagostena, L. (1998) A discriminant block among K⁺ channel types by phenytoin in neuroblastoma cells. *Br J Pharmacol* 124: 1698–1702.

Pal, S., Sun, D., Limbrick, D., Rafiq, A. and De Lorenzo, R.J. (2001) Epileptogenesis induces long-term alterations in intracellular calcium release and sequestration mechanisms in the hippocampal neuronal culture model of epilepsy. *Cell Calcium* 30: 285–296.

Palma, E., Ragozzino, D., Di Angelantonio, S., Mascia, A., Maiolino, F., Manfredi, M. et al. (2007) The antiepileptic drug levetiracetam stabilizes the human epileptic GABAA receptors upon repetitive activation. *Epilepsia* 48: 1842–1849.

Patsalos, P.N. (2000) Pharmacokinetic profile of levetiracetam: toward ideal characteristics. *Pharmacol Ther* 85: 77–85.

Pisani, A., Bosni, P., Martella, G., De Persis, C., Costa, C., Pisani, A. et al. (2004) Intracellular calcium increase in epileptiform activity: modulation by levetiracetam and lamotrigine. *Epilepsia* 45: 719–728.
Poo, G.P., Migliore, M. and Johnston, D. (2002) Pharmacological upregulation of h-channels reduces the excitability of pyramidal neuron dendrites.

Nat Neurosci 5: 767–774.

Potschka, H., Baltes, S. and Lüscher, W. (2004) Inhibition of multidrug transporters by verapamil or probenecid does not alter blood-brain barrier penetration of levetiracetam in rats. Epilepsy Res 58: 85–91.

Poulain, P. and Marginu, D.G. (2002) Levetiracetam opposes the action of GABA_A antagonists in hypothalamic neurones. Neuropharmacology 42: 346–352.

Ramael, S., Daoust, A., Otoul, C., Toublanc, N., Troenar, M., Lu, Z.S. et al. (2006) Levetiracetam intravenous infusion: a randomized, placebo-controlled safety and pharmacokinetic study. Epilepsy Res 47: 1128–1135.

Reding, J.C., Jahnnes, H. and Mosfeldt Laursen, A. (1990) The effect of two lipophilic gamma-aminobutyric acid uptake blockers in CA1 of the rat hippocampal slice. Br J Pharmacol 99: 103–106.

Rigolot, J.M., Hans, G., Nguyen, L., Rocher, V., Belachew, S., Malgrange, B. et al. (2002) The antiepileptic drug levetiracetam reverses the inhibition by negative allosteric modulators of neuronal GABA_A and glycine-gated currents. Br J Pharmacol 136: 659–672.

Robinson, R.B. and Siegelbaum, S.A. (2003) Hyperpolarization-activated cation currents: from molecules to physiological function. Annu Rev Physiol 65: 453–480.

Sawada, S. and Yamamoto, C. (1985) Blocking action of pentobarbital on receptors for excitatory amino acids in the guinea pig hippocampus. Exp Brain Res 59: 226–231.

Shannon, H.E., Eberle, E.L. and Peters, S.C. (2005) Comparison of the effects of anticonvulsant drugs with diverse mechanisms of action in the formalin test in rats. Neuropharmacology 48: 1012–1020.

Sills, G.J., Leach, J.P., Fraser, C.M., Forrest, G., Patalsky, P.N. and Brodie, M.J. (1997) Neurochemical studies with the novel anticonvulsant levetiracetam in mouse brain. Eur J Pharmacol 325: 35–40.

Simeone, T.A., Wilcox, K.S. and White, H.S. (2006) Subunit selectivity of topiramate modulation of heteromeric GABA(A) receptors. Neuropharmacology 50: 845–857.