# Epileptic Disorders

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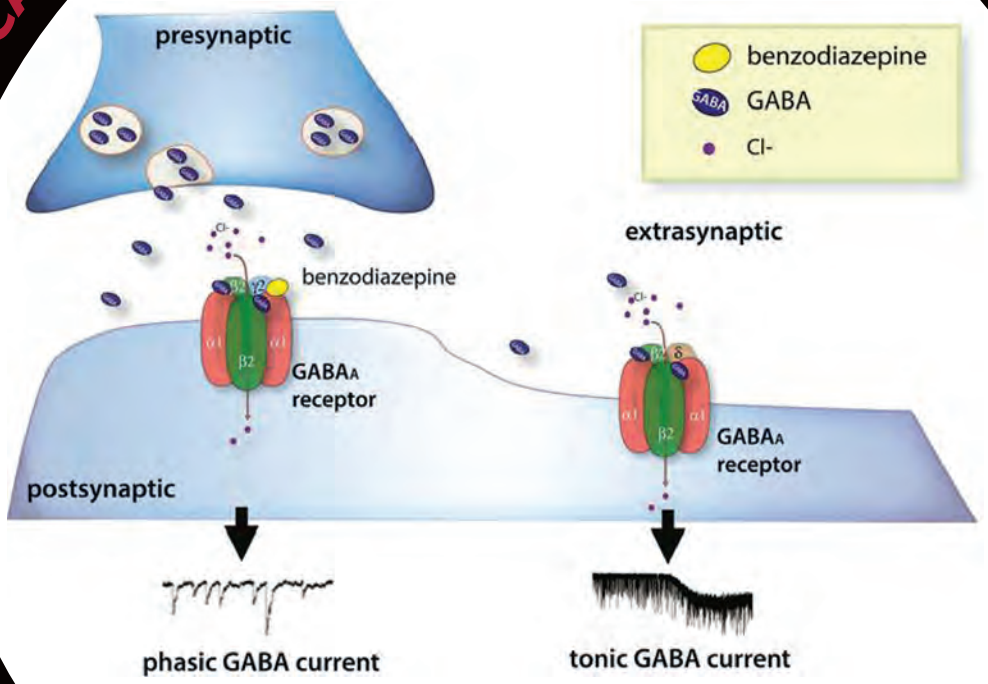
## Prolonged Epileptic Seizures: Identification and Rescue Treatment Strategies

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# Prolonged Epileptic Seizures: Identification and Rescue Treatment Strategies

***GUEST EDITORS:***

***ALEXIS ARZIMANOGLOU, THOMAS BAST, JAUME CAMPISTOL, RICHARD CHIN, ARISTEA GALANOPOULOU, LIEVEN LAGAE***

*Supplement 1, Volume 16, October 2014 to Epileptic Disorders* summarizes a *Progress in Epileptic Disorders Workshop* on **Prolonged Epileptic Seizures** that took place at *Château de Faverges*, Lyon, France, in October 10-12, 2013.

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Viropharma was not involved in the content of the symposium or preparation of the supplement; all participants actively contributed to the preparation of presentations and debates that followed.

An honorarium was not provided to speakers, chairs or other participating experts.

***Cover page figure: Courtesy Pr. Aristea Galanopoulou***

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*Guest Editors:*
*Alexis Arzimanoglou, Thomas Bast, Jaume Campistol, Richard Chin, Aristea Galanopoulou, Lieven Lagae*
*Château de Faverge, LYON, Thursday October 10th - Saturday October 12th, 2013*

## CLINICAL EXPERIENCE WITH BENZODIAZEPINES



## DEVELOPMENTAL PHARMACOLOGY OF BENZODIAZEPINES



## INDICATIONS IN CLINICAL PRACTICE



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# Understanding the mechanisms, identifying and treating prolonged epileptic seizures

The majority of convulsive generalized tonic-clonic seizures terminate prior to two minutes with a small percentage prolonged up to five and occasionally ten minutes.

Which are the factors leading to an occasional seizure prolongation? Which factors involved to seizure termination fail, consequently allowing some of the seizures to continue and develop into status epilepticus? What are the mechanisms involved?

How can children at risk for prolonged seizures can be identified and treated? Can we agree upon an operational definition of prolonged seizures to determine optimal timing of treatment to prevent established status epilepticus?

Understanding of the mechanisms underlying the predisposition for prolonged seizures may lead to improved management pathways. The contributions published in this supplement of *Epileptic Disorders* are the final product of constructive debates held during an international experts workshop devoted to the topic of prolonged seizures, particularly in children, covering:

- Definition of what constitutes a prolonged seizure and how long to delay before administering rescue medication
- Consequences of prolonged seizures on human brain
- Lessons learned from basic science and animal models
- EEG and Neuroimaging aspects
- Benefits and risks from early treatment;
- Overtreatment; Management in children and adults
- A comparison of available treatments, stability in the conditions of emergency, drug absorption and Guidelines for out of hospital management
- Suggestions for future research and clinical trials

The workshop covered all these aspects in detail (see the individual manuscripts) , and at the end, all participants agreed on some clinically very relevant practical guidelines. Intravenous benzodiazepines remain a first step for the in hospital treatment of prolonged seizures or status epilepticus. However, in the community, in a pre-hospital situation, intravenous administration is not possible. In recent years, it was shown that rectal, buccal, intranasal, and intramuscular administration of benzodiazepines is very effective as a first and safe treatment step. In many cases, rectal diazepam is not socially acceptable anymore.

The prescription of rescue medication remains an individual decision and the question as to whether or not it should be prescribed for an individual patient depends on many factors. All experts agreed upon the fact that the most important risk factor for SE is the history of a previous prolonged event. Therefore, rescue medication is recommended for this subgroup of patients independent of the epilepsy syndrome.

Experts agreed that we certainly need more controlled prospective trials using large consortia with well-defined study criteria and end points. They also agreed upon the fact that translational research needs to be pursued aiming at a better understanding of the underlying pathogenic mechanisms.

All workshop participants insisted that the first need today remains to establish the acceptance of available therapies among those treating children. Rescue medication unavoidably involves parents, family friends and schoolteachers. It remains our responsibility to establish best practices, in close interaction with social scientists, medical professionals and lay organisations.

Currently available evidence allows us to confidently prescribe available rescue drugs when indicated. Once a child is identified as at risk, time for rescue administration has to be determined on an individual basis. The "5 minutes" represent a reasonable, and easy to teach, reference frame. The treating physician, who will also have to take into account the habitual duration of seizures of a patient, can then adapt it and appropriately advise the caregivers. □

Alexis Arzimanoglou, Lieven Lagae

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Prolonged Epileptic Seizures:
identification and treatment

*Epileptic Disord 2014; 16 (Suppl. 1): S2-S5*

# Setting the scene: definition of prolonged seizures, acute repetitive seizures, and status epilepticus. Do we know why seizures stop?

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**ABSTRACT** – Status epilepticus is recognised as an acute emergency requiring urgent intervention. The optimal timing of such an intervention during a prolonged seizure, and the reasons for such, have provided much discussion. For operational purposes, a definition of a prolonged seizure of ≥5 minutes requiring intervention appears justified. However, a definition of status epilepticus of ≥30 minutes should stand, with the proportion of seizures proceeding to this clinical state remaining small. The reasons for this may be inherent to an individual, but an understanding of the mechanisms underlying the predisposition may lead to improved management pathways in the future.

**Key words:** status epilepticus, seizure, prolonged seizure

## Historical perspective

Convulsive status epilepticus (SE) is a recognised emergency requiring urgent treatment. The definitions of prolonged seizures and status epilepticus, however, have provided much debate over many years. Early definitions referred to seizures that persisted for hours, if not often days. An early definition by Clark and Prout (1903) referred to "*a state in which seizures occur so frequently that the coma and exhaustion are continuous between the seizures*". Later, Kinnier Wilson referred to such as the severest form of seizures in which "*the post-convulsive sleep of one attack is cut short by the development of the next*" (Wilson, 1940). Later, in the initial classification of the epilepsies, the International League Against Epilepsy (ILAE) defined status epilepticus as prolonged or repetitive seizures, a situation in which "*a seizure persists for a sufficient length of time or is repeated frequently enough to produce a fixed or enduring epileptic condition*" (Gastaut *et al.*, 1964), and

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this was retained when the classification was revisited in 1970 (Gastaut, 1970). This was subsequently revised a little and defined as a condition characterised by epileptic seizures that are sufficiently prolonged or repeated at sufficiently brief intervals so as to produce an unvarying and enduring epileptic condition (Proposal, 1981).

The main criticism of the ILAE definition is that it does not define a specific duration, and for the purpose of such a definition was not recognised as requiring to do so. However, many authors have taken 30 minutes as an appropriate cut off for such a definition (Lowenstein *et al*., 1999). The rationale behind this time period was based on the fact that this is the duration of time tolerated prior to cell and neuronal death in certain animal models (Lowenstein *et al*., 1999), after which there is greater risk of decompensation both systemically and within the brain (Lothman, 1990). In practice, such a definition caters for epidemiological study, but does not indicate at which time point treatment is required. Essentially, why do seizures in most circumstances self-terminate? Why in certain circumstances is there a failure of mechanisms required to terminate a seizure? At what point is it evident that this is the case, and should we treat to try and prevent the possibility of the development of full status epilepticus? Evidence suggests that earlier intervention is likely to reduce the subsequent risk of evolution to status epilepticus (Shinnar *et al*., 2001; Chin *et al*., 2008).

## Why have treatment and who requires it?

Much of the work outlining the harm of status epilepticus originates from animal studies, notably the work of Meldrum and colleagues and their observations in baboons, as well as observational studies in humans. There has been an ongoing debate as to the effect of prolonged seizures on the hippocampus, and the possible relationship to the development of hippocampal sclerosis and later temporal lobe epilepsy (Liu *et al*., 1995; MacDonald *et al*., 1999). Meldrum determined that following induced status epilepticus of >90 minutes in baboons, neuronal alterations typical of ischaemic cell change were seen diffusely in the neocortex, the cerebellum, and the hippocampus (Meldrum and Brierley, 1973), but that acute hippocampal injury occurred in the absence of local ischaemia and hypoxia (Meldrum *et al*., 1973). Imaging studies of individuals who have experienced status epilepticus have revealed initial oedema with subsequent generalised volume loss of the brain; more specifically, deep grey matter structures appear at risk of injury. Evidence of more diffuse excitotoxic cell injury in children has been determined from post mortem studies (Tsuchida *et al*., 2007). Consequently, an operational definition is required to determine optimal timing of treatment to prevent established status epilepticus where possible.

Studies with video-EEG recordings suggest that the majority of convulsive generalised tonic-clonic seizures terminate prior to two minutes. Jenssen and colleagues evaluated 579 seizures recorded on video-EEG monitoring in 159 adults (Jenssen *et al*., 2006). All the primary and majority of secondary generalised tonic-clonic seizures terminated before 5 minutes; only two of the secondary generalised tonic-clonic seizures lasted longer than 10 minutes. In a further study, 226 prospective SE cases (91 children and 135 adults) from an ongoing epidemiological study and 81 retrospective cases (31 children and 50 adults), lasting >10 and <29 minutes, from a similar two-year period, were compared (DeLorenzo *et al*., 1999). There was no statistically significant difference in the age, gender or ethnic distribution between the two groups. In the prolonged seizure group, 42% of the seizures stopped spontaneously and patients did not receive treatment, whereas the remaining 58% received AED treatment. This was significantly different to the SE group where only a small number stopped prior to AED administration. The mortality of the prolonged seizure group that stopped seizing spontaneously was zero and only 5.8% in the treated group. For the SE group, the mortality rates were 19% and 18% for treated and spontaneous termination, respectively. Consequently, it would appear there is evidence to treat a prolonged seizure, at the very least, at 10 minutes. However, on the basis of these data, as well as the fact that it appears unreasonable to wait for treatment of an individual arriving at an emergency department, a proposal of an operational definition of >5 minutes has now been widely used for working practice (Lowenstein *et al*., 1999; National Institute of Health and Clinical Excellence, 2012). This was originally put forward for adults and older children >5 years, with >5 minutes of continuous seizures or two or more discrete seizures separated by incomplete recovery of consciousness. It was proposed that in view of the unique forms of prolonged seizures in young children and infants, especially febrile seizures, a longer time frame of 10-15 minutes was suggested, however, with the recognition that there was no available data. It would appear illogical, given the susceptibility of the immature brain, for a different definition to be implemented in this age group. It has also been proposed that there should be a separate mechanistic definition for research purposes, a condition in which there is a failure of the "normal" factors that serve to terminate typical generalised tonic-clonic seizures (Lowenstein *et al*., 1999).

## Epidemiology

Epidemiological studies have suggested an overall incidence in adults and children of 0.6-1.9/10,000 if a definition of seizure duration >30 minutes is taken (Coeytaux *et al*., 2000; Hesdorffer *et al*., 1998; Knake *et al*., 2001; Wu *et al*., 2002). The Richmond study determined an ethnic difference with an incidence of 4.1/10,000 in a large non-white population, with only 1.8/10,000 if the white population was addressed (DeLorenzo *et al*., 1996). More specifically, in children, a similar incidence of 1.45/10,000 was determined in the North London study (Chin *et al*., 2006), with an ethnically adjusted incidence of 1.15. In the latter study, febrile status and acute symptomatic aetiology were responsible for almost 50% of cases and 12% occurred in individuals with an existing diagnosis of epilepsy.

A further question arises as to the risk of a prolonged seizure being the presentation of new-onset seizures. In a study of 407 children with first unprovoked seizures, seizure duration was determined using a structured interview and review of medical and ambulance records (Shinnar *et al*., 2001). Mean duration of all seizures was 12.2 minutes; seizures lasted ≥5 minutes in 50% cases, ≥10 minutes in 29%, ≥20 minutes in 16%, and ≥30 minutes in 12%. The longer the seizure lasted, the less likely it was to stop within the next few minutes. In the 189 children with two or more seizures, the duration of the first and second seizure was highly correlated. They concluded that the seizure duration in children with a first unprovoked seizure is different to that in children with refractory epilepsy, and that a subgroup of children are predisposed to prolonged seizures. No clear relationship has been determined with duration of seizure, frequency of SE, or treatment (Raspall-Chaure *et al*., 2006; Camfield and Camfield, 2012), however, an underlying cause appears to be most significantly related (Raspall-Chaure *et al*., 2006; Stroink *et al*., 2007; Camfield and Camfield, 2012). The issue remains, therefore, as to why seizures do not terminate in certain individuals. The study discussed above suggests a susceptibility to prolonged seizures in individuals, with two or more subpopulations showing a tendency to SE (Shinnar *et al*., 2001), and further clues arise from twin studies, with concordance demonstrated in identical twins (Corey *et al*., 1998). There is also a degree of syndrome specificity with early SE, a hallmark of Dravet syndrome, as well as other *SCN1A*-related epilepsies. Notably, in this syndrome, the tendency to have prolonged seizures and SE reduces with age, suggesting a dynamic in the susceptibility (Jansen *et al*., 2006). However, with regard to pathophysiology, if a common underlying problem could be determined, novel treatments could be more targeted and consequently more successful. Several mechanisms for seizure termination have been suggested. These may involve neuronal membranes and synapses, the networks involving neurons and interneurons, and even subcortical structures moderating the balance between inhibition and excitation (Lado and Moshé, 2008).

Within a single neuron, prolonged depolarisations with sustained action potential firing may be initiated by a brief depolarising pulse, or maybe the result of sustained excitatory input. Intrinsic mechanisms of seizure termination active in a single neuron include potassium currents activated by ion entry, loss of ionic gradients, and possibly local depletion of energy substrates. At a neuronal network level, depletion of inhibitory neurotransmission (glutamate, GABA), changes in intracellular and extracellular environments, failure of gap junction decoupling, or effects induced by neuromodulators (endocannabinoids, adenosine, neuropeptide Y) have all been reported to be possibly contributory. Finally, the interrelationship between cortical and subcortical structures and the possible influence on seizure generation and propagation has to be taken into account. Computational modelling studies have suggested that there may be a critical point at which seizures continue, regardless of the mechanism discussed above by which they continue to be propagated (Kramer *et al*., 2012). Utilising human EEG data and computational modelling, Kramer and colleagues suggest human brain electrical activity at various spatial scales exhibits common dynamical signatures of an impending critical transition in the approach to seizure termination, whereas activity in SE repeatedly approaches but does not cross the critical transition. This may of course have a genetic or other aetiological basis. Notably, such susceptibility may be enhanced by resistance to antiepileptic treatment that could be inherent or induced; in one individual who died at 21 years of age following SE, having experienced seizures since seven years of age, arising from a left frontotemporal dysplasia, upregulation of drug transporter proteins was demonstrated through immunohistochemistry in both cerebral hemispheres (Sisodiya and Thom, 2003). The finding of such in the normal hemisphere suggests possible induction through the SE, and may explain, in part, super-refractory SE.

## Conclusion

Over time, there appears to have been little questioning as to the definition of status epilepticus; a seizure or series of seizures that last for 30 minutes or more without consciousness gained between seizures. Data also suggest that the use of an operational definition is justified for the treatment of prolonged convulsive seizures lasting five minutes or more. The issue

remains, however, as to why seizures do not terminate in certain individuals, and whether study of the mechanisms underlying the failure to terminate seizures may result in novel treatments. Further questions remain, not least: do young children justify a relaxation of the operational definition, how we may better define the relative role of determined mechanisms responsibly for seizure cessation, and how this may be translated to clinical practice. □

**Disclosures.**
Professor Cross sits on Advisory Boards for Viropharma, Eisai, and GW Pharma, and was on the steering group for the PERFECT study. Remuneration was made to her department.

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Prolonged Epileptic Seizures:
identification and treatment

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# What are the effects of prolonged seizures in the brain?

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**ABSTRACT** – Convulsive status epilepticus is the most common neurological emergency in children and is associated with significant morbidity and mortality. The morbidities include later development of epilepsy, cognitive impairment, and psychiatric impairments. There has been a long-standing hypothesis that these outcomes are, at least in part, a function of brain injury induced by the status epilepticus. There is evidence from animal models and prospective human studies that the hippocampus may be injured during febrile status epilepticus although this pathophysiological sequence remains uncommon. Potential mechanisms include excitotoxicity, ischaemia, and inflammation. Neuroprotective drugs reduce brain injury but have little impact on epileptogenesis or cognitive impairments. Anti-inflammatory treatments have given mixed results to date. Broad-spectrum anti-inflammatory agents, such as steroids, are potentially harmful, whereas prevention of leucocyte diapedesis across the blood brain barrier appears to have a positive outcome. Therefore, more studies dissecting the inflammatory process are required to establish the most effective strategies for translation into clinical practice. In addition to neuronal loss, cognitive impairments are related to neuronal re-organisation and disruption of neural networks underpinning cognition. Further understanding of these mechanisms may lead to novel therapies that prevent brain injury, but also therapies that may improve outcomes even if injury has occurred.

**Key words:** convulsive status epilepticus, seizure, cognition, hippocampus, outcome

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Convulsive status epilepticus (CSE) is the most common medical neurological emergency (DeLorenzo *et al.*, 1996; Chin *et al.*, 2006). For the purposes of this review, CSE is defined as a seizure, or series of seizures during which full consciousness does not return, that lasts for at least 30 minutes. Although shorter time definitions have also been suggested, these definitions largely apply to treatment rather than to brain injury. The incidence in childhood is 17-21/100,000 children per year in London (Chin *et al.*, 2006), 108/100,000 children/year in Kilifi (Sadarangani *et al.*, 2008), and 38/100,000 children/year in Okayama (Nishiyama *et al.*, 2007), reflecting important geographical differences in the nature of CSE. These differences are likely to be related

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to differences in aetiology of CSE. Significant mortality and morbidity has been reported. Mortality is almost exclusively in children with significant pre-existing brain abnormalities. However, morbidity, at least in part, may be related to direct effects of seizures (Pujar *et al.,* 2011). The aim of this review is to describe the relationships between CSE, brain injury, and cognitive outcomes, and to discuss potential mechanisms for these outcomes.

## CSE and hippocampal injury

There is a long-standing hypothesis that CSE, and particularly prolonged febrile seizures, are associated with hippocampal injury which gives rise to the most common justification for epilepsy surgery; mesial temporal sclerosis (MTS) (Babb and Brown, 1993; Honovar and Meldrum, 1997). This hypothesis was generated from observations made in epilepsy surgery programs *i.e.* in retrospect. More recently, there has been an emphasis on prospectively identifying children with prolonged febrile seizures (PFS) and assessing whether these children have evidence of hippocampal injury, how any observed injury matures, and how this injury may impact cognition. It is recognised that these relationships may take many years and therefore these issues have not yet been fully resolved in humans.

Animal models have widely been used to investigate whether CSE can cause hippocampal injury (Ben Ari, 1985; Lothman *et al.,* 1989; Meldrum, 1991; Cavalheiro, 1995). This has been extensively reviewed elsewhere (Lothman and Bertram, 1993; Cavalheiro, 1995) and will not be covered in detail here. In brief, there are several well-established models in which the generation of CSE, using chemical means (*e.g.* bicuculline, pilocarpine, kainate) and electrical stimulation approaches, leads to hippocampal injury that closely resembles the histological features of MTS in humans (Babb and Brown, 1993). The outcomes from these animals include spontaneous recurrent limbic seizures and cognitive impairments. These studies have been used to justify human prospective studies.

There are two main studies in the literature which have used MRI to evaluate the relationship between prolonged febrile seizures and hippocampal injury. The London group have shown that there is evidence for bilateral hippocampal oedema 48 hours after a PFS (Scott *et al.*, 2002). None of these children had visually-identified abnormalities which were identified using quantitative analysis tools (Scott *et al.*, 2002). This peak in hippocampal oedema has been confirmed in an animal model and in addition, the T2 relaxation time at 48 hours predicts the severity of the final hippocampal volume loss (Choy *et al.*, 2010a). Subsequently, these hippocampi return to normal size six months later, although there is an increase in right-left asymmetry when compared to controls (Scott *et al.*, 2003). The relationship between early T2 and final hippocampal volume was not confirmed in this cohort and it is likely that a larger sample size is required for this. No child developed MTS in that timeframe.

A subsequent study by the London group evaluated hippocampal growth over the first year following CSE. As it has been established that PFS is associated with acute hippocampal changes, the children in the next cohort were investigated 1, 6 and 12 months after CSE with the assumption that acute hippocampal oedema would have gone by one month after the event. In total, 20-30% of the children had evidence for reduction in hippocampal growth, further supporting the view that CSE may cause hippocampal injury in some children (Yoong *et al.*, 2013). This cohort included children with PFS as well as children with other forms of CSE. It is important to note that this growth failure was identified at a similar frequency across all types of CSE.

The FEBSTAT study in the USA has recruited 199 children with PFS and therefore has greater power to identify later MTS than the London studies (Scott and Neville, 2009). FEBSTAT has revealed that there are approximately 10% of children that have visually identified increases in T2 unilaterally in the hippocampus (Shinnar *et al.,* 2012). There is some suggestion that this increase in T2 can predict final volume as in our animal models (Provenzale *et al.*, 2008) and in the recently reported results from FEBSTAT (Lewis *et al.*, 2014). Therefore, it is clear that PFS is associated with acute hippocampal change and that it is possible that this leads to adverse seizure and cognitive outcomes. Understanding the mechanisms of this injury could lead to novel treatments that minimize the chances of these adverse outcomes. It is extremely difficult to establish a mechanism and therefore animal models are required.

The mechanism that has received the most attention is excitotoxicity (Meldrum, 1991; Haglid *et al.*, 1994). Prevention of brain injury and epileptogenesis is possible with pre-administration of the NMDA receptor blocker, MK-801, supporting the hypothesis that excitotoxicity is an important mechanism driving adverse outcomes from CSE (Stafstrom *et al.*, 1993). Unfortunately, administration of MK-801 after termination of CSE, which is the more clinically relevant experiment, fails to prevent epileptogenesis even though brain injury is lessened. This suggests that either the mechanisms downstream of NMDA receptor activation or modulators of those mechanisms could be potential therapeutic targets. The time course of oedema identified in both humans and animal models is consistent with an inflammatory process, and given that the degree of oedema is related to the severity of brain

injury, it is possible that modulation of inflammation could improve outcomes from CSE.

There is increasing evidence that CSE in rodents elicits brain inflammation and that blocking inflammatory cascades can improve outcomes. CSE induced electrically, with convulsant drugs or with high temperatures, results in rapid activation of glial cells and concomitant production of inflammatory molecules. Interleukin-1β is induced within one hour of CSE and is observed in the area of seizure origin (Dhote *et al*., 2007; Ravizza *et al*., 2008). The release of this and other cytokines (*e.g*. tumour necrosis factor [TNF] and interleukin-6 [Il-6]) (Vezzani and Granata, 2005; Dubé *et al*., 2005; Vezzani *et al*., 2011) results in up-regulation of selectins, adhesion molecules (including vascular cell adhesion molecule-1 [VCAM-1]) (Jung *et al*., 2006; Fabene *et al*., 2008) and integrins (Fabene *et al*., 2008). These latter molecules allow the rolling and arrest of leukocytes along the endothelium and subsequently enable the transmigration of those leukocytes across the endothelium. These processes are believed to be important in the modulation of brain injury and epileptogenesis. Global gene expression studies in animal models of CSE and traumatic brain injury have shown prominent up-regulation of immune response genes at multiple time points from the acute insult. If inflammatory processes are modulating the mechanisms underpinning brain injury and epileptogenesis, then the fact that inflammation continues throughout the time course of injury and epileptogenesis makes inflammatory molecules very attractive therapeutic targets. It is also possible to image evidence of inflammation using a contrast agent targeted to VCAM-1 (Duffy *et al*., 2012). Therefore, it is clear that there is a relationship between CSE and inflammation, although it remains uncertain whether these changes are causatively related to brain injury and adverse outcomes.

The pharmacological experiments that have attempted to address the relationships between inflammation and adverse outcomes from CSE have explored the effects of cyclooxygenase-2 (COX-2) inhibitors, erythropoietin, disruption of leukocyte-endothelial interactions, and corticosteroids. There is controversial evidence that reducing inflammation following CSE with COX-2 inhibitors can reduce the severity of subsequent epilepsy. Administering the COX-2 inhibitor celecoxib following CSE reduces the severity of hippocampal injury and the frequency of spontaneous recurrent seizures in the pilocarpine model (Jung *et al*., 2006). The COX-2 inhibitor parecoxib also reduces the severity of brain injury, but does not alter the frequency or duration of spontaneous recurrent seizures when administered following pilocarpine-induced CSE (Serrano *et al*., 2011). However, the severity of the seizures is reduced.

Erythropoietin is known to have neuron and astroglial protective effects *via* several mechanisms including the reduction of tissue-injuring molecules, such as reactive oxygen species, glutamate, and inflammatory cytokines. Administration of erythropoietin for seven days, commencing immediately after termination of status epilepticus, reduces hippocampal injury as well as the frequency and severity of subsequent spontaneous recurrent seizures (Chu *et al*., 2008; Jung *et al*., 2011). This suggests that a broad spectrum anti-inflammatory agent has positive effects on outcomes from CSE. Another broad spectrum anti-inflammatory is dexamethasone. When this is administered soon after CSE and then daily for five days, brain injury is greater than in controls with CSE, and mortality is greater (Duffy *et al*., 2014). Thus, a greater understanding of the inflammatory mechanisms to disentangle advantageous from disadvantageous processes may provide insight on treatment.

The fourth approach that has been tested is the disruption of leukocyte-endothelial interactions. CSE leads to up-regulation of VCAM-1, which is important in the rolling and arrest of leukocytes. This effect is mediated by P-selectin glycoprotein ligand-1 (PSGL-1, encoded by Selplg) and leukocyte integrins $\alpha 4\beta 1$ and $\alpha_L\beta 2$. Genetically interfering with PSGL-1, using blocking antibodies to $\alpha 4$, and depleting leukocytes all result in reduced brain injury and reduced epileptogenesis (Fabene *et al*., 2008). This suggests an extremely important role for leukocyte vascular interactions in injury and epileptogenesis.

Another possible mechanism of brain injury is related to blood flow. During pilocarpine-induced status epilepticus, there are increases in blood flow across many parts of the brain. This is hypothesised to be in order for the brain to meet the metabolic demand of the seizure, thereby minimizing brain injury. However, the increase in blood flow to the hippocampus does not increase as much as in the cortex, despite the hippocampus having marked epileptic changes (Choy *et al*., 2010b). Thus, there is a relative hyporaemia in the hippocampus which may, at least in part, lead to hippocampal injury. It is probable that there is no one predominant mechanism of injury and injury is likely to be a result of an interaction between all of the processes described as well as possible mechanisms not yet described.

## Cognitive outcomes

Brain injury associated with status epilepticus could result in long-term cognitive impairment. The London group has been exploring this issue and have shown that one month following CSE, there are reductions in cognitive and language abilities (Martinos *et al*., 2013).

One year following CSE, there were no further changes to cognition, suggesting that the identified cognitive impairments were fixed within one month of the event. These findings are confounded by the aetiology of the CSE which is also a major driver of impairment and therefore the outcomes could be a result of a pre-existing condition. Children with non-febrile CSE often have important neurological illness and have more impairment than those with febrile seizures who are usually considered to be neurologically normal. Nevertheless, children with PFS also have a reduction in cognitive and language ability when compared to controls. Although this may be a result of the mechanisms that predispose a human to having PFS, it is also possible that this reduction of approximately 10 DQ points is a direct effect of the seizure. As the hippocampus is integral to memory function, we also investigated this. Children with PFS have abnormalities in memory retention one month after PFS (Martinos *et al*., 2012).

There are many potential mechanisms underlying these observed cognitive changes. It is commonly attributed to neuronal loss and thus insufficient neural machinery to adequately process information. However, there are other potential processes that could lead to cognitive impairments. A common observation after hippocampal injury is mossy fibre sprouting in the dentate gyrus (Buckmaster *et al*., 2002). This phenomenon is thought to increase excitability of the hippocampus and thus predispose to seizures, and it is likely that this disruption of the neural system could also impair cognition. Alterations in signalling processes including AKT/MToR (Talos *et al*., 2012), as well as increased neurogenesis with abnormal migration and integration, may also contribute to cognitive outcomes (Danzer, 2008). Thus, there are many factors that could lead to adverse cognitive outcomes and the net effects of these events are likely to disrupt the neural networks underpinning cognition. Behaviourally, it has been shown that after CSE, animals have marked impairments in the Morris water maze which is a test of spatial memory in rodents.

There are several lines of evidence supporting the view that the function of neural networks is impaired following brain injury associated with CSE. Changes in long-term potentiation have been identified in hippocampal slices and this may be important as a mechanism for the marked spatial memory impairments seen following CSE-induced brain injury (Cornejo *et al*., 2007). Cognitive networks can also be interrogated *in vivo* by looking at individual cells, EEG oscillations, and functional connectivity between cells. Place cells are hippocampal pyramidal neurons that exhibit location-specific firing which can be recorded in freely moving rodents. Parameters from those recordings give insight into the fidelity with which these cells fire, *i.e*. a cell that fires very precisely with location specificity is thought to encode spatial information with high fidelity and predicts hippocampal function. Place cells are impaired following brain injury, but importantly, are also disrupted in a PFS model in which there is no overt neuronal loss (Dubé *et al*., 2009). Place cells also show the phenomenon of phase precession which describes the way a place cell fires with respect to EEG oscillations (Lenck-Santini and Holmes, 2008). This phenomenon which describes how a place cell functions within its network is also abnormal post CSE. Direct correlations between how well an animal performs in a maze and these parameters have not been attempted.

It has been recognised for decades that the frequency and size of oscillations in the theta frequency (4-12 Hz) in the rodent hippocampus are important for information processing. These oscillations are also known to fluctuate with respect to running speed. In animals with CSE-induced hippocampal injury, the speed modulation of theta frequency is less precise and the degree of imprecision predicts how well an animal performs in a figure-8 alternation task (Richard *et al*., 2013). The evaluation of EEG oscillations gives some information about neural networks. An alternative way to evaluate neural networks is to build functional connectivity networks from single unit recordings in which multiple neurons are recorded simultaneously. Following CSE-induced hippocampal injury, there is increased functional connectivity between hippocampal pyramidal cells and the level of connectivity predicts performance in the same alternation task as above (Tyler *et al*., 2012). The networks that form during the running phase of the task are expected to reactivate when the animal rests at the feeder. The level of reactivation also predicts performance in the spatial task. Therefore, spatial cognition impairments following CSE are related to the way surviving neurons are organised into networks and not simply to the number of neurons. If it were possible to modulate the networks of remaining neurons then this may lead to strategies that improve cognition.

In conclusion, status epilepticus is likely to cause some brain injury although the frequency at which this leads to MTS and epilepsy remains uncertain. Mechanisms of injury include excitotoxicity, inflammation, and relative reductions in hippocampal blood flow. There is evidence for cognitive impairment following CSE in humans and in animal models and this may be related to disruptions in the neural networks underpinning cognition. Further understanding of these mechanisms may lead to novel therapies that prevent brain injury, but also therapies that may improve outcomes even if injury has occurred. □

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**Prolonged Epileptic Seizures: identification and treatment**

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# What is more harmful, seizures or epileptic EEG abnormalities? Is there any clinical data?

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**ABSTRACT** – Cognitive impairment is a common and often devastating co-morbidity of childhood epilepsy. While the aetiology of the epilepsy is a critical determinant of cognitive outcome, there is considerable evidence from both rodent and human studies that indicate that seizures and interictal epileptiform abnormalities can contribute to cognitive impairment. A critical feature of childhood epilepsy is that the seizures and epileptiform activity occur in a brain with developing, plastic neuronal circuits. The consequences of seizures and interictal epileptiform activity in the developing brain differ from similar paroxysmal events occurring in the relatively fixed circuitry of the mature brain. In animals, it is possible to study interictal spikes independently from seizures, and it has been demonstrated that interictal spikes are as detrimental as seizures during brain development. In the clinic, distinguishing the differences between interictal spikes and seizures is more difficult, since both typically occur together. However, both seizures and interictal spikes result in transient cognitive impairment. Recurrent seizures, particularly when frequent, can lead to cognitive regression. While the clinical data linking interictal spikes to persistent cognitive impairment is limited, interictal spikes occurring during the formation and stabilization of neuronal circuits likely contribute to aberrant connectivity. There is insufficient clinical literature to indicate whether interictal spikes are more detrimental than seizures during brain development.

**Key words:** interictal spikes, cognition, learning, memory

Cognitive impairment is a devastating co-morbidity of childhood epilepsy. Many parents and clinicians consider the cognitive impairment associated with childhood epilepsy to be far more impairing than the seizures. While the primary determinant of cognitive outcome in childhood epilepsy is aetiology, there is increasing evidence that seizures and interictal EEG abnormalities contribute to cognitive impairment. A critical question is which is more detrimental, the seizures or the interictal abnormalities? Answering this question is fundamental to our therapeutic approach to children with epilepsy.

It is often difficult to differentiate the adverse cognitive effects of interictal spikes (IIS) from those of seizures since typically they occur together. Additionally, teasing out the effects of the seizures and IIS from the aetiology can be difficult. In animal studies, one can induce

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seizures, IIS, or both, in the normal brain, allowing investigation into the biological mechanisms underpinning cognitive impairment due to seizures or IIS. For the most part, in animals studies of recurrent seizures, the seizures are brief (<5 minutes in duration). In this review, pertinent animal data will first be briefly discussed laying the groundwork for the human studies.

## Animal data

### Recurrent seizures

There is now a substantial literature showing that recurrent seizures in the developing brain can result in long-term adverse consequences. Rat pups subjected to a series of recurrent brief seizures during the first weeks of life have considerable cognitive impairment including deficits of spatial cognition based on the Morris water maze (Holmes *et al*., 1998; Huang *et al*., 1999; Liu *et al*., 1999; Karnam *et al*., 2009a; Karnam *et al*., 2009b) and delayed non-match-to-sample task, a spatial memory test in which animals have to remember which of two levers to press to obtain a food award (Kleen *et al*., 2011a), impairment of auditory discrimination (Neill *et al*., 1996), altered activity level (Karnam *et al*., 2009a), and reduced behavioural flexibility (Kleen *et al*., 2011b). Recurrent early-life seizures also result in a number of physiological changes including a persistent decrease in GABA currents in the hippocampus (Isaeva *et al*., 2006) and neocortex (Isaeva *et al*., 2009), enhanced excitation in the neocortex (Isaeva *et al*., 2010), impairment in spike frequency adaptation (Villeneuve *et al*., 2000), marked reductions in after-hyperpolarising potentials following spike trains (Villeneuve *et al*., 2000), impaired long-term potentiation (LTP) (Karnam *et al*., 2009a), enhanced short-term plasticity (Hernan *et al*., 2013), alterations in theta power (Karnam *et al*., 2009b), and impaired place cell coherence and stability (Karnam *et al*., 2009b).

Despite the detrimental effects of early-life seizures on cognitive function, recurrent brief seizures during the first two weeks of life do not result in cell loss (Holmes *et al*., 1998; Liu *et al*., 1999; Riviello *et al*., 2002). However, seizures in immature rats can result in synaptic reorganisation, as evidenced by CA3 sprouting (Holmes *et al*., 1998; Huang *et al*., 1999; Sogawa *et al*., 2001; Huang *et al*., 2002) and decreased neurogenesis (McCabe *et al*., 2001).

To determine the relationship between age at seizure onset and cognitive outcome, Karnam *et al*. (2009a) induced 50 brief seizures using flurothyl, an inhaled convulsant, in rat pups between postnatal day (P) P0-10 or P15-P25. The seizures in the rats were characterised by clonic activity followed by tonic extension with a total duration of <5 minutes. Rats were studied as adults in the Morris water maze, radial-arm water maze, open field, and active avoidance test. To assess synaptic strength and network excitatory and inhibitory function, animals were evaluated with long-term potentiation (LTP) and paired-pulse facilitation/inhibition. Compared to controls, both groups of rats with recurrent seizures were impaired in spatial memory in both water maze tests and had altered activity in the open field. Rats with recurrent flurothyl seizures had impaired LTP but showed no deficits in paired-pulse facilitation or inhibition. The cognitive deficits did not vary as a function of age during which time the seizures occurred.

Whereas recurrent brief flurothyl-induced seizures in immature rats result in cognitive impairment, recurrent seizures in adult animals, in which the neuronal circuitry is relatively fixed, appears to result in fewer deficits. Investigators have examined the effect of kindling on spatial memory in animals which were studied after or during kindling using both the radial arm maze and water maze. The timing of the kindling stimulations determines type of deficit. If the kindling stimulation is given prior to the learning trial there is impaired performance (McNamara *et al*., 1992; Robinson *et al*., 1993; Gilbert *et al*., 2000), whereas kindling immediately after the learning trial impaired retention (Gilbert *et al*., 1996). Whether kindling has long-term effects on learning is not clear; some authors report impairment following hippocampal kindling (Leung *et al*., 1990; Leung and Shen, 1991) while other authors report no long-standing effects (McNamara *et al*., 1992). While Lin *et al*. (2009) found that recurrent flurothyl-induced seizures over 11 days in adult rats lead to progressive impairment in a spatial hidden goal task, full recovery did occur.

In the majority of studies, recurrent seizures have been induced in normal rats. However, in children, seizures do not occur in the "normal brain". The assumption that seizures induced in the normal and pathological brain have similar effects may be erroneous. Lucas *et al*. (2011) found that seizures induced in rat pups with malformations of cortical development, but without seizures, had severe spatial cognitive deficits based on the water maze. When the rat pups were subjected to recurrent flurothyl-induced seizures and tested at 25 days of age (immediate post-weaning), there was a worsening of performance. In contrast, in animals tested during adolescence, there was no longer an additional adverse effect of seizures. The authors also investigated whether the severity of the structural abnormality and seizures impacted brain weight, cortical thickness, hippocampal area, and cell dispersion area. Early-life brief seizures did not have a significant impact on any of these parameters. These

observations indicate that the major factor responsible for the cognitive impairment in the rats with cortical dysplasia was the underlying brain substrate, not the seizures.

**Interictal spikes**

In adult rats, IIS have been shown to result in task-specific cognitive impairment. Using a within-subject analysis to analyse how IIS might independently affect memory processing in the hippocampus, Kleen *et al*. (2010) studied rats that developed chronic IIS following intrahippocampal pilocarpine in a hippocampal-dependent operant behaviour task, the delayed match-to-sample test. Hippocampal IIS that occurred during memory retrieval strongly impaired performance. However, IIS that occurred during memory encoding or memory maintenance did not affect performance in those trials. IIS were most dysfunctional when hippocampal function was critical, during the active engagement of neurons involved in performing the task.

Single-cell firing patterns have been investigated following IIS in mature rodents. There is a sustained reduction of action potentials in the hippocampus for up to two seconds following IIS. Furthermore, when occurring in flurries, IIS can reduce action potential firing for up to six seconds (Zhou *et al*., 2007). The widespread inhibitory wave immediately after IIS can also reduce the power of gamma oscillations and other oscillatory signals in the hippocampus (Urrestarazu *et al*., 2006). Since oscillations are closely coupled with ongoing learning and memory function (Halasz *et al*., 2005), this disruption in oscillations likely contributes to cognitive deficits.

In addition to causing transitory cognitive impairment, IIS during early brain development may have long-term adverse effects on the developing neural circuits. In studies of the effects of IIS on network development, IIS were elicited by either penicillin (Baumbach and Chow, 1981; Crabtree *et al*., 1981) or bicuculline (Ostrach *et al*., 1984; Campbell *et al*., 1984) through focal application on the striate cortex of rabbits. IIS were elicited for 6-12 hours following each drug application which was given daily from P8-9 to P24-30. Despite frequent IIS, none of the rabbits had behavioural seizures. In single-unit recordings from the lateral geniculate nucleus, superior colliculus, and occipital cortex ipsilateral to the hemisphere with IIS, there was an abnormal distribution of receptive field types, whereas normal recordings were found from the contralateral hemisphere. Remarkably, this finding was age-dependent. Adult rabbits with similarly induced IIS during adulthood had normal disruption of receptive field types, highlighting an additional vulnerability of critical developmental periods to cumulative IIS effects over time.

To determine the long-term effects of IIS on executive function, Hernan *et al*. (2014) studied the effects of IIS in the prefrontal cortex. P21 rat pups received intracortical injections of bicuculline into the prefrontal cortex while the EEG was continuously recorded and the animals were tested as adults for short-term plasticity. At the time the adults were tested, IIS were no longer present. IIS resulted in a significant alteration in short-term plasticity bilaterally in the prefrontal cortex. In a delayed non-match-to-sample task, the rats showed marked inattentiveness without deficits in working memory. Rats also demonstrated deficits in sociability, showing autism-like behaviour. The study showed that early-life focal IIS in the prefrontal cortex have long-term consequences for cognition and behaviour at a time when IIS are no longer present. This study also showed that focal IIS during development can disrupt neural networks, leading to long-term deficits and thus may have important implications in attention deficit disorder and autism.

Generalised and multifocal IIS have also been elicited in young rats with flurothyl (Khan *et al*., 2010). Rat pups were given a low dose of flurothyl for four hours for a period of ten days during continuous EEG monitoring. Rats developed IIS without seizures while age-matched controls under similar testing conditions showed few IIS. When rats were tested as adults, there was impairment in reference memory in the probe test of the Morris water maze, reference memory impairment in the four-trial radial-arm water maze, and impaired LTP. Early-life IIS also resulted in impaired new cell formation and decreased cell counts in the hippocampus, indicating a potential mechanism in which IIS during development can produce cumulative lasting effects in addition to any dynamic disruptions.

**Lessons from the animal data**

Animal data indicates that recurrent seizures and IIS can result in adverse effects on cognition. Both seizures and IIS can result in transient cognitive impairment. In the case of seizures, the transient cognitive impairment occurs during the seizure and postictal period, whereas IIS specifically alters the neural circuits involved in that process, stressing the importance of matching the affected neural substrate with a cognitive test that assesses its intrinsic function. The IIS must occur at a particular moment in cognitive processing such that the process is vulnerable to disruption.

Both seizures and IIS in the immature brain can have permanent adverse effects on cognition that extend

well beyond the time when the seizures and IIS have stopped. Both seizures and IIS appear to be deleterious when they occur in the developing brain, relative to the fully mature neural network.

## Human data

### Seizures

Animal data would predict that recurrent seizures in the immature brain, particularly if very frequent, would result in cognitive impairment. This appears to be the case in children. In general, childhood epilepsy carries a significant risk for a variety of problems involving cognition. The distribution of IQ scores of children with epilepsy is skewed toward lower values (Farwell *et al*., 1985; Neyens *et al*., 1999), and the number of children experiencing difficulties in school because of learning disabilities or behavioural problems is greater than in the population without epilepsy (Williams *et al*., 1998; Sillanpaa *et al*., 1998; Wakamoto *et al*., 2000; Bailet and Turk, 2000). Predictors of poor cognitive outcome include a high seizure frequency (Hermann *et al*., 2008) and long duration of the epilepsy (Farwell *et al*., 1985; Seidenberg *et al*., 1986).

However, many children that develop epilepsy appear to have cognitive deficits that precede the onset of the seizures, suggesting that aetiology of the seizures, and not the seizures themselves, are responsible for the impaired cognition (Berg *et al*., 2004; Fastenau *et al*., 2009; Jackson *et al*., 2013). Most children with epilepsy maintain stable IQ scores. However, there is evidence that some children with epilepsy have delayed mental development (Neyens *et al*., 1999) or even have progressive declines of IQ on serial intelligence tests over time (Bourgeois *et al*., 1983; Berg *et al*., 2004).

In a community-based cohort, 198 children, aged <8 years with new-onset epilepsy, were followed prospectively and reassessed using the Wechsler Intelligence Scales for Children (WISC) 8-9 years later (Berg *et al*., 2012). The authors found that pharmacoresistant epilepsy was associated with an 11.4-point lower full scale IQ. It was found that in the absence of pharmacoresistance, age was not associated with cognitive scores. Although the initial level of adaptive function on the Vineland Adaptive Behavior Scale (VABS) was correlated with later cognitive function, it did not account for the impact of pharmacoresistance on later function. The impairment observed in the children with pharmacoresistant epilepsy involved multiple cognitive subdomains of the WISC, in particular verbal comprehension and perceptual organisation.

In the case of temporal lobe epilepsy in children, increasing duration of epilepsy is associated with declining performance across both intellectual and memory measures (Hermann *et al*., 2002). In a study of 46 children and adults (age range: 14-59 years) with temporal lobe epilepsy, a cognitive trajectory that differed from age- and sex-matched healthy controls was reported (Hermann *et al*., 2006). Adverse cognitive outcomes were observed in approximately a quarter of the patients, particularly in memory.

Animal data would also suggest that epilepsy onset in early childhood is detrimental. Indeed, predictors of cognitive impairment in children with epilepsy include early onset of seizures (Huttenlocher and Hapke, 1990; Glosser *et al*., 1997; Bulteau *et al*., 2000; Bjornaes *et al*., 2001; Hermann *et al*., 2002; Cormack *et al*., 2007), particularly during the neonatal period (Glass *et al*., 2009). Studies have demonstrated correlations between IQ and age at onset in a variety of refractory childhood-onset epilepsies treated surgically (Vasconcellos *et al*., 2001; Jonas *et al*., 2004; Cormack *et al*., 2007; Vendrame *et al*., 2009; D'Argenzio *et al*., 2011) or pharmacologically (O'Callaghan *et al*., 2011). Investigators have demonstrated that earlier intervention, especially for seizures beginning in infancy, results in better developmental outcomes and the ability to rebound after surgery (Jonas *et al*., 2004; Freitag and Tuxhorn, 2005; Loddenkemper *et al*., 2007).

Children with epileptic encephalopathies have cognitive impairment at the onset of epilepsy and also have significant declines over time. The epileptic syndromes in which psychomotor deterioration occurs exhibit an early age at onset. Such syndromes include early infantile epileptic encephalopathy with suppression-burst (Ohtahara syndrome), early myoclonic encephalopathy, migrating partial epilepsy in infancy, infantile spasms (West syndrome), severe myoclonic epilepsy of infancy (Dravet syndrome), Lennox-Gastaut syndrome, myoclonic-astatic epilepsy, continuous spike-wave discharges of slow wave sleep (CSWS), and Landau-Kleffner syndrome (LKS) (Genton and Dravet, 1997; Panayiotopoulos, 2002; Nabbout and Dulac, 2003).

While aetiology of the epilepsy undoubtedly plays a major role in cognitive development, early-life seizures independent of aetiology can lead to cognitive impairment (Glass *et al*., 2009; Korman *et al*., 2013). In a study of neuropsychological function in children with focal cortical dysplasia, Korman *et al*. (2013) found that age at onset of epilepsy and extent of the dysplasia each contributed independently to cognitive dysfunction. The authors suggested that early onset of epilepsy disrupts critical periods of development and leads to poor cognitive outcomes. Furthermore, it was concluded that a later age at onset of epilepsy would not be expected to mitigate deficits because of widespread pathology, nor would

a localised lesion be likely to mollify the developmental deficits resulting from an early age at epilepsy onset.

**Interictal spikes**

Animal studies would predict that IIS result in transitory cognitive impairment. IIS in humans can produce brief disturbances in neural processing, resulting in a phenomenon called "transitory cognitive impairment" (Binnie, 2003). Aarts *et al*. (1984) noted that IIS can briefly disrupt neural processes affecting function within the brain region where they occur. The authors analysed the effect of IIS on verbal or non-verbal short-term memory in patients with epilepsy but without overt clinical manifestations during these discharges, thus targeting the so-called "subclinical" manifestations of IIS. In right-handed individuals, the authors reported that right-hemisphere IIS were associated with errors in a non-verbal task whereas left-hemisphere IIS resulted mainly in errors in verbal tasks. EEG discharges interfered mainly when they occurred simultaneously with the presentation of the stimulus, corresponding to the encoding phase of the task. Shewmon and Erwin in a series of elegantly performed studies (Shewmon and Erwin, 1988a; Shewmon and Erwin, 1988b; Shewmon and Erwin, 1988c; Shewmon and Erwin, 1989) further localised the effect, noting that occipital IIS could disrupt visual perception. IIS in the occipital region caused transitory deficits with stimuli presented in the contralateral visual field. Deficits were most pronounced when the stimulus was presented during the slow wave following the IIS.

In a study of 10 adult patients with depth electrodes implanted into their hippocampi for preoperative seizure localisation, Kleen *et al*. (2013) recorded EEG during 2,070 total trials of a short-term memory task, with memory processing categorised into encoding, maintenance, and retrieval. The influence of hippocampal IIS on these processes was analysed and adjusted to account for individual differences between patients. Hippocampal IIS occurring in the memory retrieval period decreased the likelihood of a correct response when they were contralateral to the seizure focus ($p<0.05$) or bilateral ($p<0.001$). Bilateral IIS during the memory maintenance period had a similar effect ($p<0.01$), particularly with spike-wave complexes of longer duration ($p<0.01$). The results strengthen the view that IIS contribute to cognitive impairment in epilepsy depending upon when and where they occur. The results of this study confirmed an earlier study by Krauss *et al*. (1997) who found declines in working memory due to IIS.

Because of their frequent nature, IIS in benign epilepsy with centro-temporal spikes (BECTS) has generated considerable interest. The vast majority of studies have found that children with BECTS have a variety of cognitive impairments (Fonseca *et al*., 2007a; Danielsson and Petermann, 2009). Children with BECTS have been reported to have mild language defects, revealed by tests measuring phonemic fluency, verbal re-elaboration of semantic knowledge, and lexical comprehension (Riva *et al*., 2007; Verrotti *et al*., 2011), as well as impairment in non-verbal functions (Metz-Lutz *et al*., 1999; Metz-Lutz and Filippini, 2006). The cognitive profile of the deficits is related to the side of focus with non-verbal deficits significantly correlated with the lateralisation of the epileptic focus in the right hemisphere with verbal deficits observed with left hemisphere discharges. Frontal functions, such as attention control, response organisation, and fine motor speed, were impaired in the presence of active discharges independently of the lateralisation of the epileptic focus (Metz-Lutz *et al*., 1999; Metz-Lutz and Filippini, 2006). However, not all studies have shown consistent neuropsychological profiles in children with BECTS. Some of the variability in function may be explained by fluctuations in IIS frequency and cognitive performance. In a study of six children with BECTS, month-to-month marked fluctuations in cognitive abilities and frequency and location of IIS have been noted (Ewen *et al*., 2011).

Transitory cognitive impairment has been studied during IIS in children with BECTS using EEG and computerised neuropsychological testing with a word and pseudoword visual discriminating task (Fonseca *et al*., 2007b). A small percentage of children (15.4%) made a significantly greater proportion of errors during IIS than during IIS-free periods. Of interest, in this study, the IIS were inhibited by the task, likely due to increased alertness, in 20 of the 33 children.

Whether there is a relationship between the frequency of IIS and cognition is unclear; some authors report a relationship between the number of spikes (Filippini *et al*., 2013) and others report no such relationship (Fonseca *et al*., 2007a; Tedrus *et al*., 2010; Goldberg-Stern *et al*., 2010). In a study of IIS in 182 children with a variety of epilepsy syndromes, including BECTS, Ebus *et al*. (2012) calculated the IIS index using a 24-hour ambulatory EEG and compared the findings to neuropsychological tests. The IIS index was calculated in wakefulness and in sleep, as percentage of time in five categories (0%, <1%, 1-10%, ≥10-50%, and ≥50%). The group of patients with diurnal IIS in ≥10% of the EEG record showed impaired central information processing speed, short-term verbal memory, and visual-motor integration. This effect was observed independently of other EEG-related and epilepsy-related characteristics, as well as epilepsy syndrome diagnosis.

If IIS can cause cognitive impairment, it would be reasonable to consider suppressing the IIS with antiepileptic drugs. In a double-blinded, placebo-controlled, crossover study, 61 children with well-controlled or mild epilepsy were randomly assigned to add-on therapy with either lamotrigine followed by placebo, or placebo followed by lamotrigine (Pressler *et al*., 2005). Global rating of behaviour significantly improved only in patients who showed a significant reduction in either frequency or duration of discharges during active treatment, but not in patients without a significant change in discharge rate. However, in a small study using sulthiame to treat the IIS in BECTS, it was found that children had a significant deterioration in their reading ability, despite a reduction in IIS frequency (Wirrell *et al*., 2008). A major obstacle to designing studies to treat IIS is the lack of well tolerated drugs that effectively suppress IIS.

Despite the impairment observed during the presence of active IIS, children with BECTS have no permanent effects of the IIS, with the vast majority of children having no residual cognitive impairment (Callenbach *et al*., 2010). However, two related conditions which appear to be a continuum of BECTS, LKS and CSWS, have a substantially worse prognosis (Halasz *et al*., 2005; Mikati and Shamseddine, 2005; Metz-Lutz and Filippini, 2006; Margari *et al*., 2012; Seegmuller *et al*., 2012).

LKS is a rare childhood disorder characterised by a loss or regression of previously acquired language and epileptiform discharges, involving the temporal or parietal regions of the brain (Landau and Kleffner, 1957; Cooper and Ferry, 1978; Hirsch *et al*., 1990; Beaumanoir, 1992). Although a considerable amount of variation exists in the disorder, the typical history is of a child developing an abrupt or gradual loss of language ability and inattentiveness to sound, with onset during the first decade of life. This interruption in communication skills is generally closely preceded, accompanied, or followed by the onset of seizures or an abnormal EEG, or both (Sawhney *et al*., 1988; Deonna, 1991). Receptive dysfunction, often referred to as verbal auditory agnosia (Rapin *et al*., 1977), may be the dominant feature early in the course of the disorder. In some children, the disorder progresses to a point at which the child cannot even recognise sounds. In addition to the aphasia, many of the children have behavioural and psychomotor disturbances, often appearing autistic.

The EEG in LKS typically shows repetitive spikes, sharp waves, and spike-and-wave activity in the temporal region or parietal-occipital regions, bilaterally. Sleep usually activates the discharge, and, in some cases, the abnormality is observed only in sleep recordings. Speech deficits in the syndrome may be explained by either disruption of normal connections or an excessive inhibitory reaction to epileptiform discharges. However, the severity of the aphasia does not always have a close correlation with degree of EEG abnormality (Foerster, 1977; Holmes *et al*., 1981) or clinical seizures (Landau and Kleffner, 1957). It has been suggested that the epileptiform activity is an epiphenomenon and simply is reflective of an underlying cortical abnormality (Lou *et al*., 1977; Kellermann, 1978; Holmes *et al*., 1981). Even if the EEG parallels speech recovery, this does not prove that epileptiform activity causes aphasia. It is also possible that the decreased epileptiform activity during speech recovery simply reflects resolving injury to the speech areas.

While steroid treatment and intravenous immunoglobulin have been shown to be effective in treating LKS (Mikati and Shamseddine, 2005), this could be used to treat the underlying cause of LKS, such as inflammation. However, there is limited data indicating that there is a direct relationship between IIS and language impairment. Subpial resection, which eliminates epileptiform activity in the receptive language cortex, has been shown to reduce IIS and resolve linguistic function in LKS (Grote *et al*., 1999; Castillo *et al*., 2008). Since subpial resection would not be expected to alter the underlying aetiology of LKS, the fact that the patients improve with a destructive surgical procedure would indicate that the epileptiform discharges contribute LKS.

A condition related to LKS is epilepsy with CSWS (Tassinari *et al*., 2000). The disorder has also been called *electrical status epilepticus during sleep* (ESES). The distinguishing feature of CSWS is the continuous bilateral and diffuse slow spike-wave activity persisting through all of the slow-wave sleep stages. The spike-wave index (total minutes of all spike-waves multiplied by 100 and divided by the total minutes of non-REM sleep without spike-wave activity) ranges from 85 to 100%. The cause of CSWS is unknown, but early developmental lesions play a major role in approximately half of the patients, and genetic associations have recently been described. Clinical, neurophysiological, and cerebral glucose metabolism data support the hypothesis that interictal epileptiform discharges play a prominent role in the cognitive deficits by interfering with the neuronal networks at the site of the epileptic foci but also at distant connected areas (Van, 2013). High-dose benzodiazepines and corticosteroids have been successfully used to treat clinical and electroencephalographic features (Sanchez Fernandez *et al*., 2013a; Sanchez Fernandez *et al*., 2013b). As with LKS, there is no definitive data that indicates that the EEG abnormalities are responsible for the cognitive impairment. However, as with LKS, children with CSWS typically do not improve unless there is a reduction of spike-wave discharges during sleep (Scholtes *et al*., 2005; Brazzo *et al*., 2012).

There also appears to be a link between IIS and autism. Studies examining the EEG of individuals with autistic spectrum disorder show a very high rate of IIS (Hashimoto *et al*., 2001; Kim *et al*., 2006; Parmeggiani *et al*., 2007). For example, Hughes and Melyn (2005) found abnormal EEGs with IIS in 75% of 59 children with childhood autism. Many children with ASD have IIS on their EEG but do not experience seizures (Kim *et al*., 2006). In children with ASD, the most common location of IIS is in the frontal region, suggesting that frontal dysfunctions are important in the mechanism of symptoms in autism (Hashimoto *et al*., 2001). The location of IIS in the frontal regions is of interest since one of the major abnormalities in children with ASD is a disturbance in executive control (Hughes *et al*., 1994; Hughes *et al*., 1997; Hughes *et al*., 1999). The prefrontal cortex is a critical structure likely to be involved in executive control (Bachevalier and Loveland, 2006; Dumontheil *et al*., 2008; Shalom, 2009).

In children with ASD, it is not clear whether epileptiform discharges contribute or cause ASD, or whether ASD is a disturbance of brain function and epileptiform discharges are a reflection of a dysfunctional brain. In this regard, the rodent data is of interest in view of the finding that IIS in the prefrontal cortex of rats results in ASD-like behaviour (Hernan *et al*., 2013).

## Which is more harmful: interictal spikes or seizures?

There is now clear evidence that both seizures and IIS in immature rodents and children can result in cognitive impairment. The effects of both IIS and seizures in the immature brain are dependent upon brain maturation. In the fully developed brain, seizures and IIS result in temporary impairment and appear to have few long-term effects, whereas in the developing brain, both IIS and seizures have more profound effects.

Determining which is worse, seizures or IIS, is difficult to determine clinically since it is difficult to separate out the two. It is widely believed that frequent epileptiform events observed in children with epilepsy are capable of causing deleterious alterations in developing brain networks and are therefore associated with the high incidence of cognitive deficits and psychiatric comorbidities in these patients. □

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# Prolonged seizures: what are the mechanisms that predispose or cease to be protective? A review of animal data

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**ABSTRACT** – There is no doubt that seizures change processes in neuronal networks which themselves impact on seizure susceptibility, and reports on such changes probably account for the majority of studies in experimental epileptology. As much as there is no doubt about this general fact, there is, to date, quite some disagreement on whether such changes are pro-epileptic, anti-epileptic, or both, and which are crucial and which are less so. While it is not possible to provide a general answer to this, this review attempts to categorise and highlight some of these findings, and relate them to specific ontogenetic or pathophysiological conditions. Data from studies of animal models (nearly exclusively) is presented, with a focus on two main aspects: ontogenetic particularities and pathophysiological conditions, supporting evidence of susceptibility and seizure termination mechanisms in adult animal models.

**Key words:** prolonged seizures, predisposition, seizure termination, ontogenesis, modeling, adenosine, GABA, metabolism, neuromodulators, ion channels, ionic currents

Seizures occur upon changes in the brain and its function; this is a generally accepted fact. It is also widely accepted that some, if not most, of these changes impact on seizure susceptibility, or even on the mechanisms which determine seizure duration and termination. These changes have been the subject of a number of reviews on the mechanisms of seizure generation, particularly in the ontogenetically-immature brain (Holmes and Ben-Ari, 2001; Holmes *et al.*, 2002; Heinemann *et al.*, 2002; Avanzini and Franceschetti, 2003; Avoli *et al.*, 2005; Brooks-Kayal, 2005; Löscher and Köhling, 2010); these cited reviews only represent a small sample. Apart from the general problem of differentiating between the chicken and the egg (*i.e.* causative changes and epilepsy, or vice versa), these changes may act in homeostatic or even protective ways. This review will focus on two main issues,

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ontogenetic particularities and pathophysiological conditions supporting evidence of susceptibility and seizure termination mechanisms in adult animal models, while only briefly touching on protective mechanisms and status epilepticus (SE)-associated functional alterations as predisposing factors to subsequent seizures.

## Seizure-induced changes

There is a plethora of functional and structural changes associated with seizures, ranging from neuronal loss (from location- and neuron type-specific, to general), sprouting and network reorganisation, to alterations of voltage-gated or ligand-gated ion channels and receptors. Arguably, for many of these, it is difficult to differentiate whether they are a consequence or primary cause of seizures (other than those that are genetically determined), as many are considered to be detrimental by increasing seizure propensity or inflicting further functional damage (Sutula *et al.,* 2003; Löscher and Brandt, 2010). In contrast, some may actually limit seizures or act in a neuroprotective fashion (Lado and Moshé, 2008). Below, the mechanisms involved in seizure predisposition or prevention will be reviewed.

### Mechanisms that prevent seizures

In adult animal brain tissue, after single or prolonged seizures, a number of changes have been reported which either specifically affect changes in seizure threshold or more broadly result in preconditioning and neuroprotective actions.

### Seizure threshold changes

While prolonged seizures, in particular SE, in tissues of adult animals, generally result in subsequent development of spontaneous recurrent seizures (as in pilocarpine- or kainate-induced SE), as first described by (Turski *et al.,* 1983) and (Ben-Ari and Lagowska, 1978; Ben-Ari *et al.*, 1979), single and brief seizures may have opposite effects, at least transiently. Increases in seizure threshold after previous seizures, known since the 1940s (Toman *et al.,* 1946), have systematically been analysed by the group of Nutt *et al.* (1981); for an overview see Löscher and Köhling (2010). To summarise these reports briefly, in different models (kindling, maximal electroshock, and others), a previous, usually single seizure, or a series of mild seizures will at least transiently increase seizure-induction threshold for subsequent seizures, lasting from minutes to several hours, whereas this is not the case for subconvulsive attacks. As also reviewed by Löscher and Köhling (2010), changes in GABA transmission may play a role, albeit resulting in both up- (receptor density) and down-regulation (presynaptic release) (Tuff *et al.,* 1983; Löscher and Frey, 1987; Swijsen *et al.,* 2012). In the case of early-life seizures, these effects may even be long-lasting (Swijsen *et al.,* 2012). Furthermore, evidence from transcriptome analyses demonstrate down-regulation of calcium signalling, including subunits of voltage-gated calcium channels, and neuronal excitability components, including NMDA- and AMPA-receptor subunits (Jimenez-Mateos *et al.,* 2008), which could also account for an increase in seizure threshold. At least in immature animals, there are also conflicting reports suggesting an increase in excitability and a reduction in seizure threshold (Gashi *et al.,* 2007). Summarising these findings, in mature tissue, *single or mild seizures* appear to have short- (hours) to longer-term (days) effects, resulting in transiently reduced excitability which is likely due to changes in inhibitory, as well as excitatory, transmission and voltage-gated channels. In immature tissue, some evidence points to increased excitability, an issue which will further be discussed below.

### Neuroprotection

It is highly conceivable that a reduction in excitability, as discussed above, can also lead to a protection from cell death, *i.e.* neuroprotection, as calcium signalling is also linked to excitotoxicity. This injury protection (coined "epileptic tolerance") was proven in a variety of models, as reviewed by Jimenez-Mateos and Henshall (2013), including the kindling model (Kelly and McIntyre, 1994; Andre *et al.*, 2000; Penner *et al.*, 2001) and electroshock (Kondratyev *et al.*, 2001) and kainic acid-induced seizures (Blondeau *et al.,* 2000). Again, these findings are not undisputed; anti-apoptotic protection may be provided, however, it may also fail, even after single seizures, and induce damage (Bengzon *et al.,* 1997; Andre *et al.,* 2000). It remains to be tested whether distinct models (electroshock *vs.* amygdala kindling; *i.e.* deleterious *vs.* beneficial; [Andre *et al.*, 2000]) or kindling positions (amygdala *vs.* hippocampal; *i.e.* beneficial *vs.* deleterious; [Bengzon *et al.,* 1997; Andre *et al.,* 2000 respectively]) actually account for these disparate results.

### Predisposing mechanisms

In contrast to the main tenor of the previous section, the consequences of seizures may also result in increased seizure predisposition and excitability, in particular, as long-term (days and months) rather than short-term effects of initial, and severe, insults on the

one hand, and under specific circumstances in juvenile tissue on the other. These findings will briefly be summarised.

### During ontogenesis

Most findings in neonatal and infant tissue (in rats this age ranges from postnatal day 0 to 17 [P0-P17]) suggest that early-life seizures, or more specifically SE, have little impact on subsequent seizure susceptibility, or even neuronal injury or network changes (as reviewed by Scantlebury *et al*. [2007]), even though SE can be more severe than in adults. In prepubescent rodents (P18-P30), this relative protection against subsequent damage declines, and full vulnerability is reached in adults (Scantlebury *et al.,* 2007). Under certain circumstances, however, subsequent increases in seizure susceptibility have also been reported in immature animals. Whether increased excitability is induced or not may either depend on the epilepsy model used or an underlying pathology. Regarding underlying pathologies, the conversion of relatively resistant tissue to tissue that becomes more seizure-susceptible, and more excitable, appears to result from both artificially induced migration disorders or cortical dysplasia and neonatal hypoxia, possibly due to interference with GABAergic inhibition or voltage-gated currents, such as $I_h$, a hyperpolarisation-activated inward current which is down-regulated after early-life hypoxia (Jensen *et al.,* 1992; Germano and Sperber, 1997; Germano *et al.,* 1998; Jensen *et al.,* 1998; Scantlebury *et al.,* 2004; Zhang *et al.,* 2006). Regarding the epilepsy model, febrile seizures appear to be another exception: If rodents experience these early in life (P8-P11), they will develop increased hippocampal excitability, again due to increased $I_h$ (Chen *et al.*, 2001; Brewster *et al.,* 2002), possibly mediated *via* hyperthermia-induced hyperventilation and associated alkalinisation, as well as cannabinoid receptor up-regulation (Chen *et al.,* 2001; Brewster *et al.,* 2002; Schuchmann *et al.,* 2006). In addition, a reduction of GABA-mediated inhibition (Liebregts *et al.,* 2002; Swijsen *et al.,* 2012) may be involved. The effect is dependent on duration of the febrile condition; more severe effects are observed if the temperature rise lasts for more than an hour, compared to around 25-30 minutes. Under prolonged febrile conditions, inflammation, as evidenced by increases in interleukin-1ß, may also play a role (Dube *et al.,* 2010). Even at later developmental stages, *i.e.* at P21, a febrile seizure episode increases responsiveness to epileptogenic agents, as well as induced cognitive dysfunction (Wilhelm *et al.,* 2012).

Importantly, although SE early in life does not increase sensitivity to convulsants (Nehlig *et al.*, 2002), other models of infantile epilepsy (at age P6-13; PTZ-induced recurrent seizures, kainate SE) do induce changes in receptor expression and function later in life. Such changes can be interpreted as increases in net excitability induced by early-in-life seizures. Thus, adult up-regulation of NMDA-receptors (Gashi *et al.,* 2007), down-regulation of the GluR2 subunit (which would convey a reduced calcium permeability to the AMPA receptor if present in the receptor molecule) (Zhang *et al.,* 2004), and even gender-specific effects on GABAergic signalling (GABA-reversal potential) (Galanopoulou, 2008) were observed.

### During the adult state

While there appears to be some consensus that in juvenile epilepsies at least two conditions, a second underlying pathology and febrile seizures, predispose to subsequent seizures in adulthood, it is much less clear which of the manifold changes associated with chronic epilepsy are actually causal for subsequent seizures (and therefore support the disease and its progression), and which are perhaps even homeostatic reactions to the seizures.

Obviously, based on genetic models of epilepsy, we can deduce that some functional changes cause subsequent seizures, with the general cautionary note that usually the whole transcriptome of such animals is not known, and consequently it remains uncertain whether compensatory processes might play pivotal roles in addition. Such defects (and their relation to human "mutants") have been extensively reviewed by Noebels (2003) and Lerche *et al*. (2013). In short, they include mutations in: voltage-gated sodium channels (prolonged opening, reduced inactivation, but also exclusive down-regulation in inter-neuronal populations), voltage-gated potassium channels (again with channel defects in principal or interneurones, or ontogenetically bound), voltage-gated calcium channel compartments (pre-synaptic down-regulation resulting in selective transmitter release changes, favouring rhythmic discharges and increases in thalamic currents), transmitter release machinery (reduction of inhibitory transmitter release, sustained release, or even alterations of co-release of *e.g.* $Zn^{2+}$), GABAergic function (GABA synthesis, receptor down-regulation or changes in deactivation kinetics, reduction in late GABA responses, and alterations in chloride distribution leading to depolarising GABA), glutamatergic function (increased calcium permeability, impaired glutamate reuptake), cholinergic and serotonin receptors, and finally proteins controlling proliferation or migration *etc*. (all reviewed in Noebels [2003], Lerche *et al*. [2013], and Lerche *et al*. [2001]). Not all of the mutations generated in animals that result in epileptic phenotypes, however, are applicable to humans.

Reports on transcriptional or post-translational changes that play a role in disease progression in

animal models usually focus on two conditions: febrile seizures early in life and post-SE mesial temporal lobe epilepsy (TLE) (Lerche *et al.,* 2013), which can therefore be considered as the two main factors favouring the emergence of increased seizure susceptibility and disease progression, as reported in animal models (McCloskey and Scharfman, 2011) and TLE patients (Walker *et al.,* 2002). Since febrile seizures have already been discussed in the previous section, in the following, the focus will be on post-SE TLE models.

In post-SE models, long-term changes in expression and post-transcriptional changes of a variety of genes have been described, among them up-regulation of juvenile forms of NMDA-receptor subunits, differential expression and splicing of AMPA-receptor subunits, down-regulation of GABA-receptors or differential $GABA_A$-receptor subunit expression, dysregulation of chloride transporters and GABA-synthesizing enzymes (glutamate decarboxylase), down-regulation of calcium-activated potassium (SK) channels, up-regulation of $I_h$, down-regulation of sodium channel subunits, up-regulation of calcium channels mediating T-type currents, up-regulation of synaptotagmin subtypes, nitric oxide (NO)-synthase and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and DNA methylation changes, which either influence net excitability (*e.g.* neuronal bursting, synaptic synchronisation) or promote cell death (Babity *et al.,* 1997; DeLorenzo and Morris, 1999; Gilby *et al.,* 2005; Porter *et al.,* 2006; Becker *et al.,* 2008; Chuang *et al.,* 2010; Barmashenko *et al.,* 2011; Schulz *et al.,* 2012; Müller *et al.,* 2013; Ryley *et al.,* 2013). The cited papers (ibid) and other studies (Macdonald and Kapur, 1999; Shao and Dudek, 2004; Shao and Dudek, 2006; Chen *et al.,* 2011) also generally provide evidence of functional sequelae of these genetic changes, such as: faster desensitization, depolarisation, or reduction in the frequency of GABA currents (regarding mini IPSC); reduced SK-mediated after-hyperpolarisations; and increased sodium-channel, $I_h$ and T-type calcium channel-dependent bursting, *etc*. Beyond this, there is a discussion as to whether cell death (Andre *et al.,* 2000) and stem cell proliferation (Parent *et al.,* 1997; Parent *et al.,* 2006) are major consequences. Moreover, these may also be epileptogenic factors at least for TLE, and further give rise to deafferentation of interneurons (dormant basket cells; a controversial subject) (Sloviter, 1991; Bernard *et al.,* 1998), a reorganisation of interneuronal wiring (Andre *et al.,* 2001), and, importantly, a reduction of intrinsic antiepileptic mechanisms such as control of adenosine-mediated excitability *via* increased adenosine degradation (Fedele *et al.,* 2005) or receptor desensitization (Hamil *et al.*, 2012).

A causal relationship between disease progression and these changes is epistemologically impossible, but it is tempting to speculate that such prolonged changes are somehow involved in progression of seizure severity in animal models (McCloskey and Scharfman, 2011). Even beyond the discussion of the chicken and the egg, it is, however, improbable that any single mechanism is key to chronification of the epileptic condition, especially since studies so far have identified a large number of divergent mechanisms.

## Seizure termination mechanisms

When addressing the question of predisposing mechanisms to prolonged seizures, rather than looking at processes which aggravate seizures (as in the previous section B), one may also ask which factors lead to seizure termination, and consequently fail once a seizure does not stop but continues to develop into SE. Studies focusing specifically on seizure termination mechanisms, interestingly, are rare, and hence most of the hints from animal studies are indirect. Nevertheless, numerous hypotheses have been put forward, which will be discussed in this section, and an overview of which is given in *table 1*.

Theoretical considerations are perhaps useful in setting the scene. In one study in which the question of why some seizures stop and others evolve into SE was decidedly addressed, human EEG, electrocorticogram (ECoG), and local field potential and multi-unit recordings were actually used (Kramer *et al.,* 2012). In this study, three distinctive features were used to characterise the phases of seizure termination: a decrease in power (and frequency), an increase in temporal correlation as well as spatial correlation, and flickering (alternating high or low variances of spectral power). The authors concluded that all seizures terminated as a result of a discontinuous critical transition from one attractor to another, *i.e.* ictal to post-ictal state. All data from SE, in turn, displayed repetitive periods of strong correlation and anti-correlation of spectral power and temporal correlation measures; in other words, with SE, the system approaches but fails to cross the boundary condition repetitively (Kramer *et al.*, 2012). This suggests that seizure termination is a sudden, and likely time-dependent, and perhaps even deterministic, rather than gradual process, as already corroborated by other modelling studies using animal absence model data (Suffczynski *et al.,* 2006). The studies also suggest that it is unlikely that a single mechanism, such as intracellular calcium accumulation, is responsible, as speculated in another modelling study (Kudela *et al.,* 2003). Rather, the theoretical considerations suggest that different mechanisms can converge into the same transition border state (Kramer *et al.*, 2012).

**Table 1.** Overview of possible endogenous mechanisms of seizure termination reported in the literature, indicating: whether parameters related to these mechanisms have been reported to change during or after seizures/epileptiform activity (functionally significant activity-dependent changes); the role in the generation of seizures/epileptiform activity determined by exogenous application or blockade of endogenous action (role in seizure initiation or maintenance); an assessment on their likely role in seizure termination. Antiepileptic actions are given in italics and proepileptic actions in bold italics.

| Possible mechanism | Functionally significant activity-dependent change | Role in seizure initiation or maintenance | Likely role in seizure termination |
|---|---|---|---|
| **Metabolic compromise** | | | |
| Glucose deprivation | No | Yes[1]<br>blocks epileptiform activity with artificial strong reduction (not in vivo) | No |
| Hypoxia, ATP deprivation | No | Yes[2,3]<br>induces seizures in ATP-sensitive K+-channel KO model | No |
| **Synaptic mechanisms** | | | |
| Glutamatergic failure | Yes<br>*Transient vesicular depletion* | Yes[4,5]<br>*Determines inter-burst intervals/duration* | Uncertain |
| GABAergic up-regulation | Yes<br>*Increase of recurrent inhibition (after few seizures)*<br>***Induction of high-frequency oscillations and epileptogenesis***<br>***Synchronisation of network activity***<br>***Depolarising actions (in chronic models / epilepsy)*** | Yes[6-14]<br>?[6]<br>***Induces mirror foci***<br>***Generates rhythmic activity***<br>***Drives bursting neurons*** | Uncertain |
| Loss in neuronal gap junction coupling | ?<br>Loss of coupling during seizures uncertain | Yes[15-18]<br>Blockade usually leads to reduction of discharges (not in all models) | Uncertain |
| **Cellular excitability** | | | |
| Potassium current activation ($Ca^{2+}$-activated/voltage-gated) | Yes<br>*Neuronal hyperpolarisation:*<br>*Reduction of burst frequency* | Yes[19,20]<br>*Determines inter-burst intervals and neuronal firing rates*<br>Changes are only short-lived and transient | ***Likely*** |
| Reduction of input resistance | Yes<br>Persistent reduction of resistance in chronic epilepsy + dynamic reduction with $K^+$-current activation: *reduction of synaptic efficacy*<br>Decrease in time constant:<br>***Increase in maximal firing rate*** | Possible[20-23] | ***Likely*** |

**Table 1.** *(Continued).*

| Possible mechanism | Functionally significant activity-dependent change | Role in seizure initiation or maintenance | Likely role in seizure termination |
|---|---|---|---|
| **Ionic environment** | | | |
| ↑extracellular $K^+$ | Yes<br>*Neuronal depolarisation block: Reduction in neuronal firing and synaptic transmission* | Yes[21,24,25]<br>***Medium levels induce seizures***<br>*High levels block neuronal activity*<br>Depolarisation block insufficient to stop network activity<br>Extracellular $K^+$ levels lower in chronically epileptic tissue than in normal one | ***Likely*** |
| ↓extracellular $Ca^{2+}$ | Yes<br>*Modulates transmitter release*<br>***Depolarises neurons (surface charge effect)*** | Yes[25-27]<br>***Initiates epileptiform activity at very low levels*** | Uncertain |
| **Glial function** | | | |
| Disturbed astrocytic $K^+$ regulation (see also Ionic microenvironment) | Yes<br>***Loss of Ba2+ sensitivity of $[K^*]_o$ due to astrocytic $K_{ir}$ down-regulation***<br>***Reduced connexin expression (in blood-brain-barrier dysfunction)*** | Possible[51] | ***Likely*** |
| **pH** | | | |
| ↓pH | Yes<br>*Intra- and extracellular acidification: reduces glutamatergic transmission, gates acid-sensing channels* | Yes 28-32<br>↑ *$CO_2$ blocks seizures* | ***Yes*** |
| **Neuromodulators** | | | |
| ↑Adenosine | Yes<br>*Activates $K^+$ and inhibits $Ca^{2+}$ channels via A1 receptors* | Yes[33-36]<br>Endogenous release controls seizure initiation and duration | ***Yes*** |
| ↑NPY | Yes<br>*Receptors up-regulated;*<br>*Reduced glutamatergic synaptic transmission via Y2 receptors* | Yes[37-39]<br>*Endogenous release controls recurrent excitation and epileptiform activity* | ***Yes*** |
| ↑Cytokines | Yes<br>*Interleukin-1Ra (endogenous receptor antagonist) reduces excitability*<br>***Interleukins (1ß, 6): increase neuronal excitability*** | Yes[40]<br>***Induces seizures***<br>*Blocks seizures* | Uncertain |
| ↑Opioids | Yes<br>Receptors up-regulated.<br>*μ-receptor-mediated reduction of neuronal excitability*<br>***μ-receptor- and BDNF mediated increase in excitability*** | Yes[41-45]<br>*Application blocks seizures;*<br>*Endogenous dynorphin expression controls seizure threshold, duration*<br>***Induce seizures*** | Uncertain |

**Table 1.** *(Continued).*

| Possible mechanism | Functionally significant activity-dependent change | Role in seizure initiation or maintenance | Likely role in seizure termination |
|---|---|---|---|
| Endocannabinoid Release | ? Release during seizures uncertain | Yes[46-50] *Exogenous endocannabinoids block seizures* ***Endogenous endocannabinoids regulate GABA-release*** ***Exogenous endocannabinoids activate glutamate release* via *TRPV1 receptor*** | Uncertain |

[1]Kirchner *et al*. (2006); [2]Namba *et al*. (1989); [3]Yamada *et al*. (2001); [4]Staley *et al*. (1998); [5]Jones *et al*. (2007); [6]Tuff *et al*. (1983); [7]Khalilov *et al*. (2003); [8]Köhling *et al*. (2000); [9]Köhling *et al*. (1998); [10]Khazipov and Holmes (2003); [11]Cohen *et al*. (2002); [12]Barmashenko *et al*. (2011); [13]Bragin *et al*. (2009); [14]Pathak *et al*. (2007); [15]Gigout *et al*. (2006); [16]Roopun *et al*. (2010); [17]Köhling *et al*. (2001); [18]Wallraff *et al*. (2006); [19]Schulz *et al*. (2012);[20]Timofeev *et al*. (2004); [21]Bikson *et al*. (2003a); [22]Stegen *et al*. (2009); [23]Isokawa (1996); [24]Pinto *et al*. (2005); [25]Lux *et al*. (1986); [26]Bikson *et al*. (2003b); [27]Cohen and Fields (2004); [28]Somjen (1984); [29]Xiong *et al*. (2000); [30]Velisek *et al*. (1994); [31]Caspers and Speckmann (1972); [32]Schuchmann *et al*. (2006); [33]Lewin and Bleck (1981); [34]During and Spencer (1992); [35]Young and Dragunow, (1994);.[36]Dunwiddie and Masino (2001); [37]Vezzani *et al*. (1999); [38]Marksteiner *et al*. (1989); [39]Tu *et al*. (2005); [40]Vezzani *et al*. (2002); [41]Koepp *et al*. (1998); [42]Hammers *et al*. (2007); [43]Loacker *et al*. (2007); [44]Avoli *et al*. (1996b); [45]Zhang and Ko (2009); [46]Karler *et al*. (1986); [47]Wada *et al*. (1973); [48]Wada *et al*. (1975); [49]Isokawa and Alger (2005); [50]Bhaskaran and Smith (2010);[51]Heinemann *et al*. (2012).

One tempting hypothesis to explain seizure arrest is a proposed depletion of resources, under the presumption that *e.g*. oxygen and/or glucose supply would drop under continued seizure activity, and hence also intracellular ATP (Doman and Pelligra, 2004). Indeed, in both chronically epileptic human tissue, as well as tissue from post-SE rats, induced seizure-like activity results in reduced NAD(P)H recovery, suggesting mitochondrial respiratory chain or glycolysis failure (Kann *et al*., 2005). While this explains ictal hypometabolism, it is unlikely, however, that it actually is instrumental in stopping seizures, precisely because, in these models, seizure-like activity actually progresses. Indeed, the authors speculate that the reduction of NADH production could be instrumental in developing pharmacoresistance (Heinemann *et al*., 2002). Furthermore, although hypoxia (and particularly re-oxygenation after hypoxia) can induce seizures, and likewise severe hypoglycaemic conditions (while moderate reductions are actually pro-convulsant) (Kirchner *et al*., 2006), local cerebral glucose utilisation is generally reduced immediately postictally in kindled rats, which suggests a lowered rather than increased glucose demand at the end of a seizure (Namba *et al*., 1989).

Loss of neuronal synchronisation *via* loss of excitatory drive or increasing impact of inhibitory mechanisms or differential function of electrical coupling is another attractive hypothesis to explain seizure termination. At first sight, experiments using an *in vitro* model of status-like activity (high $K^+$), suggesting a progressive exhaustion of presynaptic glutamate release during epileptic bursts, favour this hypothesis (Staley *et al*., 1998). However, again, in this model, inter-burst intervals successively increase due to this mechanism, but the activity, as such, persists, and burst duration actually increases with reduced and desynchronized glutamate release (Jones *et al*., 2007). The role of glutamatergic failure in seizure termination is thus uncertain. Increasing inhibitory restraint might also play a role in seizure arrest.

The concept of surround inhibition playing a role in spatial containment of seizures was already put forward by David Prince and Joe Wilder in the 1960s (Prince and Wilder, 1967). Direct recordings from interneurones from such foci demonstrate inhibitory cells to be very active during discharges (Domann *et al*., 1991). Irrespective of this initial restraint (Trevelyan *et al*., 2006; Trevelyan *et al*., 2007; Schevon *et al*., 2012), however, there are strong indications that, with the progression of seizures, or more precisely with the spatial spread of activity, the inhibitory restraint fails, as reviewed by Trevelyan and Schevon (2013).

There may be several reasons for this, including a depolarising block, presynaptic inhibition of GABA release, GABAergic vesicular depletion or postsynaptic desensitisation, and in fact also development of a depolarising drive for GABA as chloride accumulates (Dzhala *et al*., 2010); reviewed by Trevelyan and Schevon (2013). Hence, at least in acute models of epilepsy, the role of inhibition is probably a restraining one at first, but then changes to one which likely merely shapes the structure of an ictal event (*i.e*. support of tonic-like phases *vs*. clonic-like ones) without,

however, playing a role in determining their duration (Swartzwelder *et al.,* 1988). Indeed, GABA, by being rhythmically released during seizure-like activity, may actually subserve network synchronisation by pacing bursts, both in acute *in vivo* animal models (Khazipov and Holmes, 2003), as well as in human epileptic tissue (Köhling *et al.,* 1998). Beyond this, in chronically epileptic tissue, the aforementioned chloride accumulation may chronify as well, as GABAergic activity, at least in the hippocampus in TLE models and in human tissue, is often depolarising, likely due to a dysregulation in chloride transporter expression (Köhling *et al.,* 2000; Cohen *et al.,* 2002; Pathak *et al.,* 2007; Bragin *et al.,* 2009; Barmashenko *et al.,* 2011). In the worst case, GABAergic activity can finally also contribute to the expansion and chronification of the epileptic condition itself, since GABAergic fast oscillations are apparently able to establish mirror foci under certain conditions (Khalilov *et al.,* 2003; Le Van Quyen *et al.,* 2006). Phasic $GABA_A$ transmission is hence not a realistic candidate mechanism limiting seizure duration.

The case is less clear for tonic GABAergic activity, which is stable or even increased in chronically epileptic tissue (Walker and Kullmann, 2013). Whether this up-regulation of tonic current is mediated *via* neurosteroids, as in non-epileptic tissue (Stell *et al.,* 2003), remains to be elucidated, but can be disputed on the grounds that neurosteroid-sensitivity in epileptic animals *in vivo* is reduced (Lawrence *et al.,* 2010). At any rate, a reduction in the inhibitory action of phasic GABA release, combined with an up-regulation of a tonic action, is actually likely to increase the gain of neurons, and hence their responsiveness to excitatory input (Walker and Kullmann, 2013).

With respect to neuronal and/or glial gap junctions, the studies so far favour the notion that inter-neuron coupling supports synchronisation, while inter-glia coupling reduces it. The evidence comes from experiments using pharmacological or genetic blockade, which either leads to increased activity in the case of glial gap junction knockout (due to loss of spatial potassium buffering and increased extracellular potassium) (Wallraff *et al.,* 2006) or to a decrease in activity when gap junctions are pharmacologically blocked (putatively then also, or even mainly, neuronal ones) (de Curtis *et al.,* 1998; Köhling *et al.,* 2001; Gigout *et al.,* 2006; Roopun *et al.,* 2010). It would be critical to demonstrate an activity-dependent loss of neuronal gap junctional coupling or increase of glial coupling to support gap-junction involvement in shaping seizure duration. Although changes in the level of intracellular pH and calcium are activity-dependent (de Curtis *et al.,* 1998) and could well induce functional changes in gap junctions, a direct demonstration of these effects remains to be confirmed. Hence, the impact of gap-junctional coupling in seizure termination remains uncertain. Regarding ephaptic interactions, *i.e.* transmembranous currents induced by extracellular currents due to resistance changes in extracellular vs. membrane compartments, these could also possibly influence synchronisation (Köhling *et al.,* 2000). However, as there is cell swelling during seizures (Lux *et al.,* 1986), it is likely to increase, rather than to decrease at the end of seizures, and hence can probably be ruled out as a termination mechanism.

A possible seizure termination mechanism could also be a dynamic change in intrinsic neuronal excitability or transmembranous currents. The main class of transmembranous currents which reduce neuronal excitability consists of various potassium currents. Of the multitude of these, those currents which show activity-dependent activation would be particularly interesting in this context. Indeed, at least in an *in vivo* model of spike-and-wave discharges, in particular, calcium-activated potassium currents appear to limit activity duration (Timofeev *et al.,* 2004). As calcium is known to accumulate intracellularly during seizures, this mechanism could be a plausible one. Interestingly, in chronically epileptic tissue (post-SE TLE), this current appears to be critically reduced, both regarding function and expression. This predisposes the tissue to prolonged discharges (Schulz *et al.,* 2012). Input-resistance changes have been implicated in determining epileptogenicity, as they decrease in animal models and human epileptic tissue (Isokawa, 1996; Stegen *et al.,* 2009). In effect, this means that synaptic currents need to be much larger to change neuronal membrane potential, and hence, excitatory drive will be less efficient. In turn, however, this also means that firing frequency will be increased as a consequence of a decrease in time constant (remember that $\tau=RC$) (Bikson *et al.,* 2003a). More importantly, input resistance often dynamically decreases rather than increases in the course of a seizure, since further channels open and the cells become leaky, making this too a possible mechanism to stop the seizure (Timofeev *et al.,* 2004).

Among the different dynamic changes during seizures and epileptiform activity, alterations in ionic microenvironment, including pH, are well documented. A comprehensive review by Hans-Dieter Lux, Uwe Heinemann and Irmgard Dietzel summarises these phenomena (Lux *et al.,* 1986). During activity, in the focus, extracellular potassium rises to a ceiling of 12-14 mM; further increase is prevented by glia buffering. This trans-glial potassium flux is compensated, albeit not fully, by sodium, resulting in focal extracellular sodium increases, but a decrease in osmolarity,

and hence cell swelling. Calcium is reduced focally to 0.6 mM due to influx into neurons, while chloride follows the potassium buffering flux and is hence reduced in the focus. What does this mean for excitability? In particular, the increase in potassium will lead to a depolarisation of all cells within the focal area by an estimated 15-20 mV, which could actually lead to a depolarisation block (inactivation of sodium currents), and is speculated to support activity termination at least in acute *in vitro* models (Lux *et al.,* 1986; Bikson *et al.,* 2003a; Pinto *et al*., 2005). Although in chronically epileptic tissue, such potassium increases are generally lower than in healthy tissue (Köhling *et al.,* 1995) and potassium levels are mainly lower at the end of a seizure-like event than at the start (Avoli *et al.,* 1996a), it is likely that these changes do exert some influence on seizure duration, particularly should buffering be compromised. There are indeed indications in this direction. In human tissue from epilepsy patients, induced changes in extracellular potassium are not modified by $Ba^{2+}$, a blocker of inwardly-rectifying astrocytic potassium-currents ($K_{IR}$), and the expression of $K_{IR}$-channels was found to be down-regulated (hence at the first prerequisite for potassium buffering, astrocytic potassium uptake appears to be compromised). Furthermore, in addition, astocytic connexins in rodent brain (the second prerequisite for potassium buffering), challenged with blood-brain-barrier breakdown, are equally down-regulated (Heinemann *et al*., 2012). A critical experiment would now be to show that the duration of seizures actually is inversely correlated to the extent of potassium increases.

In contrast, the decrease in extracellular calcium plays an ambiguous role; it increases excitability by reducing surface charge, leading to neuronal depolarisation (Bikson *et al.,* 2003b) (something which is taken advantage of in the low-calcium epilepsy model), but also decreases epileptogenicity by reducing synaptic transmitter release (Cohen and Fields, 2004). A drop in extracellular chloride, in turn, is likely to increase excitability, since this will shift its equilibrium potential to positive, depolarising values. Having identified potassium as one possible factor of seizure termination, pH is another critical one. Thus, during seizure-like activity, the extracellular space acidifies (Somjen, 1984). Furthermore, activity duration and extracellular (Lux *et al*., 1986), as well as intracellular (Xiong *et al.,* 2000), acidification are related, and artificial acidification (also *via* $CO_2$-ventilation) stops activity both *in vitro* and *in vivo* (Caspers and Speckmann, 1972; Velisek *et al*., 1994), likely *via* several mechanisms, including activation of acid-sensing channels or interference with glutamatergic synaptic transmission (Velisek, 1998; Ziemann *et al*., 2008). Any condition supporting alkalinisation, such as hyperventilation, in turn, will prolong and exacerbate seizures (Schuchmann *et al.,* 2006). Activity-dependent acidification is hence, together with extracellular potassium accumulation, probably a critical candidate mechanism controlling seizure duration.

Activity-dependent release of neuromodulators, *i.e*. substances released as non-classic transmitters with metabotropic action, which are capable of influencing synaptic transmission, is another candidate group of seizure-terminating mechanisms. Among these, adenosine and neuropeptide Y (NPY) are most interesting. Regarding adenosine, it was shown early on that it is released endogenously in an activity-dependent fashion during seizures in animal models (Lewin and Bleck, 1981) and patients (During and Spencer, 1992). Furthermore, prolonged seizures were speculated to result from loss of adenosine function (Young and Dragunow, 1994), which is generally accepted to be net inhibitory (Dunwiddie and Masino, 2001). It is not surprising that novel therapeutic strategies are being considered on the basis of adenosine delivery (Boison, 2005). Regarding NPY, this molecule is also released during and particularly after seizures (Marksteiner *et al.,* 1989), to inhibit excitatory synaptic transmission (Tu *et al.,* 2005). In chronic epilepsy models, its release and receptor expression are up-regulated, which is interpreted as an intrinsic antiepileptic compensatory reaction (reviewed in Vezzani *et al*. [1999]).

Other potential modulators also include cytokines, endogenous opioids, and cannabinoids, however, the role of these requires further elucidation, suffice to say that cytokines are usually considered to be pro-epileptic. Yet, in chronic models, an endogenous receptor blocker of interleukin 1 receptors was also reported to be up-regulated (Vezzani *et al.,* 2002), thus one might speculate that this may also be activity-dependent. Supporting evidence for this hypothesis, however, is still lacking. Likewise, endogenous dynorphin was shown to control seizures as long as releasing fibres were conserved (Wasterlain *et al.,* 2002), presumably *via* κ receptors (Loacker *et al.,* 2007), a finding which is corroborated also in human tissue (Koepp *et al.,* 1998; Hammers *et al.,* 2007). The ambiguous nature of opioids, however, becomes evident when considering that these negatively control GABA release (Avoli *et al.,* 1996b) *via* μ receptors, and that activation of this receptor eventually leads to induction of seizures (*via* brain-derived neurotrophic factor [BDNF] expression) (Zhang and Ko, 2009). Endogenously-released cannabinoids, likewise, are ambiguous in their action, being both anti- and pro-epileptic (Wada *et al.,* 1973; Wada *et al.,* 1975; Karler *et al.,* 1986). They share with

opioids a negative control of GABA release, and beyond this they enhance glutamate release, since they cross-react also with TRPV1 channels (Isokawa and Alger, 2005; Bhaskaran and Smith, 2010). Again, more investigations are needed to gauge their net role in seizure termination.

A factor clearly supporting sustained SE apparently is Substance P (Wasterlain *et al*., 2000), which was shown to be released during seizures, and its receptors were up-regulated in the chronic epileptic condition. The unfortunate combination of release and receptor up-regulation may allow for a vicious cycle to be initiated, which is speculated to maintain SE.

### Outlook

In an attempt to simplify the interpretation of the findings discussed in this review, which probably reflect a broad consensus, the main factors derived from animal experiments which predispose to prolonged seizures are febrile seizures early in life (and possibly also later), as well as migration disorders/cortical dysplasia, or neonatal hypoxia. This emphasises the point that ontogenetic factors are paramount. The only other factor which apparently also plays an important role in determining seizure susceptibility, and perhaps also prolonged seizures, is a history of SE itself, and perhaps traumatic brain injury (Holmes *et al*., 2002), again, mainly in an ontogenetic context. Of the underlying mechanisms determining seizure duration, extracellular increases in potassium, acidification, adenosine, NPY, and substance P are interesting candidates, and future key experiments should establish whether defects in these mechanisms can be found both in animal models and the clinical context which specifically relates to prolonged seizures.

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**Prolonged Epileptic Seizures: identification and treatment**

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# Outcome of status epilepticus. What do we learn from animal data?

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**ABSTRACT** – Status Epilepticus (SE) is a life-threatening neurologic disorder defined as 5 minutes or more of a continuous seizure. SE can represent an exacerbation of a preexisting seizure disorder, the initial manifestation of a seizure disorder, or an insult other than a seizure disorder. In humans, there are several differences between SE that occurs in adults and children. In adult patients, the mortality is high but the incidence is lower than in childhood. Experimental studies have been essential in helping clinicians describe SE, and since these early initial studies, further experimental studies have helped us to better understand the consequences of SE. Animal models of SE support the notion that SE induces brain damage and contribute to epileptogenesis. Laboratory models of SE in developing animals demonstrate age- and model-dependent propensity for brain injury and for epileptogenesis. The use of models with a double hit including a clinical relevant component to seizures provides data that allows us to further understand the contribution of early-life events in the future development of epilepsy. Using this approach, it has been shown that inflammation or a preexisting brain lesion enhance epileptogenesis in the developing brain. The use of models of SE also permits to establish that treatment to stop the seizure and/or the duration of the SE results in a decrease of SE induced cell injury. Preventing epileptogenesis remains an important goal to modify the development of comorbidities, and it still represents an area of research in need of much progress.

**Key words:** status epilepticus, seizure, animal model

Status epilepticus (SE) was defined by the ILAE in 1993 as a condition in which a single seizure, or more than one seizure, continues for >30 minutes without recovery of function/consciousness (Dodson *et al.*, 1993). Several definitions have been proposed and published over the last 50 years to define SE. One of the critical points in these definitions is the criterion of time, and two time periods corresponding to the duration of SE are usually considered: 20-30 minutes and 5-10 minutes. An operational definition has been more recently proposed using a cut-off of 5-10 minutes for prompt initiation of treatment (Lowenstein *et al.*, 1999). This definition was based on the fact that a single seizure rarely lasts for longer than 2-10 minutes. After 10 minutes

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of duration, a seizure might last for about 30 minutes (Shinnar *et al.*, 2001). Initially, the criterion of a 20-30-minute duration was based on the occurrence of neuronal damage and the initiation of systemic effects, potentially damaging the central nervous system. Several experimental studies have been essential in helping clinicians describe SE, and since these early initial studies (Lothman, 1990; Meldrum *et al.*, 1973; Nevander *et al.*, 1985), further experimental studies have helped us to better understand the consequences of SE. Our aim in this review is to firstly define what kind of animal models are helpful to further understand the consequences of SE and secondly, to discuss data from experimental studies that are helpful in our understanding of this condition.

## What is a good animal model?

Much of our knowledge of epilepsy is based on the use of animal models. Animal models in the field of epilepsy are useful for a variety of tasks: the investigation of pathophysiological mechanisms, the evaluation and development of new antiepileptic treatment, and the study of the consequences of conditions that may be concurrent with epilepsy (cognitive consequences and/or comorbidities).

Animal models should meet some criteria that have been previously suggested: (1) the animal model should exhibit similar electrophysiological correlates/patterns observed in the human condition; (2) aetiology should be similar (genetic predisposition, injury, *etc.*); (3) the proposed animal model should be scaled or reflect a similar age in humans at which time the manifestations of the epilepsy syndrome are age-specific; (4) the animal model should display similar pathologies to a human condition which exhibits specific pathological changes (*e.g.* cortical dysplasia); (5) the condition being modelled should respond to similar antiepileptic drugs; and (6) the behavioural characteristics (short- or long-term behavioural changes) should reflect behavioural manifestations observed in humans (Auvin *et al.*, 2012).

### Criteria to evaluate an animal model

- Electrophysiological correlates/patterns similar to human condition
- Similar aetiology and/or injury
- Reflect human age when the epilepsy syndrome is age-specific
- Display similar pathologies (*e.g.* cortical dysplasia)
- Respond similarly to antiepileptic drugs
- Reflect cognitive/comorbidities observed in humans

## What aspects should be modelled?

The overall incidence of SE is about 40/100,000 persons per year (DeLorenzo *et al.*, 1996). In humans, there are several differences between SE that occurs in adults and children *figure 1*). In adult patients, the mortality is high (10-25%) (Logroscino *et al.*, 2005) but the incidence is lower than in childhood (DeLorenzo *et al.*, 1996; Hesdorffer *et al.*, 1998; Chin *et al.*, 2006). The mortality in children is about 2-4% (DeLorenzo *et al.*, 1996). Regarding clinical data, it would appear that outcome is not clearly related to either duration or frequency of SE (Metsaranta *et al.*, 2004), but predominantly related to the underlying aetiology (Sillanpaa and Shinnar, 2002; Stroink, 2007; Camfield and Camfield, 2012).

The number of studies in which an attempt was made to address the risk of epilepsy after SE is limited. Moreover, the results are highly variable with an overall risk of between 15 and 27% in prospective studies, while the estimated risk in retrospective studies is reported to reach 50% (Annegers *et al.*, 1982; Maytal *et al.*, 1989). It appears that most of the children who develop epilepsy following SE have a pre-existing neurological disorder or neurological risk factor (Maytal *et al.*, 1989; Shinnar *et al.*, 1996). A role for an underlying lesion in the pathogenesis of sequelae of SE, including the occurrence of epilepsy, has been realistically suggested.

## Status epilepticus in the mature brain

In rodents, SE can be induced by injection of chemicals or by electrical stimulation. There are now several models that are well described: injection of kainic acid (Benari, 1985), injection of pilocarpine (with or without lithium) (Turski *et al.*, 1986), continuous electrical stimulation of the hippocampus (Bertram, 1997), and continuous electrical stimulation of the amygdala (Nissinen *et al.*, 2000).

In these various models, seizures initially originate from limbic structures followed by secondary generalisation. The seizures are long-lasting, progressing to SE with relatively high mortality (Stafstrom *et al.*, 1993; Nissinen *et al.*, 2000). There is a rapid alteration in neuronal networks occurring within minutes to days following SE. These changes are related to modifications of ion channel kinetics, related to membrane depolarisation and modification of protein function by post-transriptional regulation and early gene activation. The initial changes are followed by subacute changes over weeks. This step includes transcriptional events, neuronal death, and inflammation. All these processes result in anatomical changes inducing network reorganisation (mossy fibre sprouting,

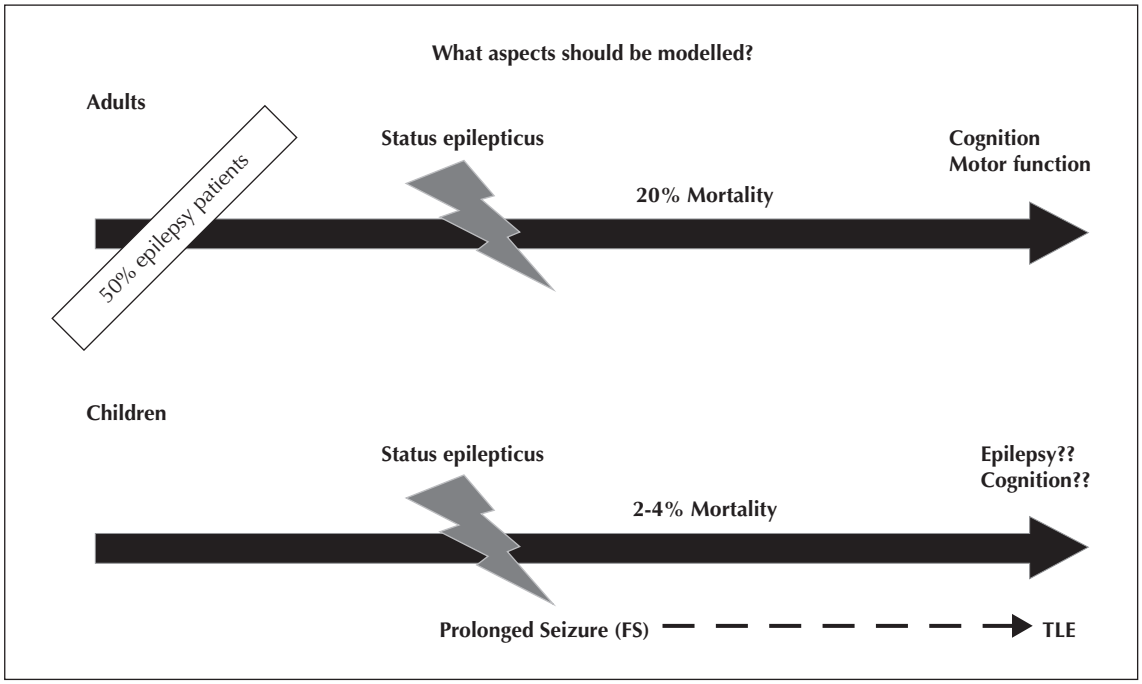


**Figure 1.** Summary of the clinical issue of status epilepticus in children and adults.

gliosis, neurogenesis, *etc.*). All these phases lead to the development of spontaneous recurrent seizures (SRS) (Stafstrom *et al.*, 1993; Priel *et al.*, 1996; Nissinen *et al.*, 2000). These different steps of epileptogenesis are extensively studied in order to understand the role of the various factors leading to SRS. This may therefore provide a basis with which to develop a new therapeutic approach to prevent epileptogenesis and discover biomarkers in order to identify patients who are at risk of developing epilepsy (Rakhade and Jensen, 2009).

SE induces neuronal cell loss. The SE-induced cell injury is widespread. In the hippocampus, this is observed in CA-1, CA-3, and dentate regions. Cell injury has been also reported in the amygdala, piriform, and entorhinal cortex and thalamus (Bertram, 1997; Nissinen *et al.*, 2000). Based on experimental models of SE in adults, some studies show that the severity of the cell loss correlates with seizure duration (Benari, 1985; Covolan and Mello, 2000). In humans, there are very few reports (<20) of human pathological studies after SE (Tsuchida *et al.*, 2007). Based on imaging studies, patients generally present evidence of oedema, at least in the temporal cortex, with neuronal necrosis observed later in the same areas. These few human studies appear to support the findings of animal models of SE, showing direct excitotoxic injury in the absence of hypoxia-ischaemia.

After a latent period following the SE attack, most rats have SRS, including secondary generalised clonic seizures (Stafstrom *et al.*, 1993; Priel *et al.*, 1996; Nissinen *et al.*, 2000). The loss of neurons in the hippocampus has been suggested to play a role in SRS, in particular, the loss of inhibitory interneurons (Cossart *et al.*, 2001; Brandt *et al.*, 2003). In addition, the animals show significant learning and memory deficits (Nissinen *et al.*, 2000; Detour *et al.*, 2005). These deficits appear to be likely related to SE-induced brain damage in limbic areas.

## Status epilepticus in the immature brain

Animal studies using rodents have suggested that the developing brain is less vulnerable to SE, compared to the mature brain. There is far less cell loss and epileptogenesis following SE in models of immature rats, relative to adult rats.

Rats are commonly used in epilepsy research of the developing brain. The multitude of differences in the rate of maturation between human and animal brains are well recognised, making precise comparisons at equivalent developmental milestones between the two species a multidimensional task. Using various parameters, we use the following as a reference for maturation- seventh postnatal day (P7) to P10: term human newborn; P14-P21: toddlers to young children; P28: older children prior to puberty; P35: adolescent.

### Status epilepticus

As reported in adult rats, the administration of kainic acid (KA) or the administration of pilocarpine or

lithium-pilocarpine represents the most widely used epilepsy models of SE in immature rats (Benari, 1985; Turski *et al*., 1986).

Using KA, immature rats at P15 cannot survive at doses tolerated in adults. Systemic KA (3 mg/kg) in P15 rats did not produce a significant level of cell injury, even though severe seizures occurred (Albala *et al*., 1984; Okada *et al*., 1984). The extent of cell injury is highly variable according to the route of administration of the KA. An intracerebroventricular application of KA results in more severe damage than that given intraperitoneally (Montgomery *et al*., 1999; Humphrey *et al*., 2002). The mortality rate in the KA model of SE is a real problem. This rate is about 90% in P15 rats (Koh *et al*., 1999). Even though this mortality rate poses a problem with regards to performing an experimental study, it has been shown that 3 mg/kg of KA results in severe seizure, but no hilar or CA3 damage is observed (Okada *et al*., 1984). Although SE induced by KA in P15 rats does not induce identifiable brain injury, the rats that are subject to a new challenge by KA at P45 experience more severe brain damage and performed worse in spatial learning tasks, relative to controls (Franck and Schwartzkroin, 1984). Thus, although cellular damage may not be clearly demonstrated after kainic acid treatment at P15, one may not conclude that this treatment has had no effect on the immature brain.

In the lithium-pilocarpine model of SE, the level of cell injury is minimal before P14 (Sankar *et al*., 1998). A significant level of cell injury is observed after P14. SE-induced cell injury is maximal in CA-1 at P14, whereas only a few damaged neurons are detected in the CA3 region. At P21, both CA-1 and CA-3 regions show extensive damage (Sankar *et al*., 1998). Neuronal damage in the amygdala and dentate granule cells is also age-specific, and the vulnerability progressively increases with age (Sankar *et al*., 1998).

The occurrence of SRS after SE is variable according to the model of SE and the age at occurrence of SE *(table 1)*. Whether cell injury to the immature brain is required to induce epileptogenesis remains an active topic of debate (Dudek *et al*., 2010; Baram *et al*., 2011). In the lithium-pilocarpine model, the occurrence of SRS in adulthood is observed in 10-20% of rats that underwent SE at P14 (mostly stage 1-2 of the Racine Seizure scale) and 50% of rats that underwent SE at P21 (Sankar *et al*., 1998). More recently, a study suggested that the consequences of SE in the immature brain occurred progressively and a similar level of cognitive consequences and epileptogenesis, compared to more mature brain, was reached (Kubova and Mares, 2013). Using the lithium-pilocarpine model at P12, they reported that both the severity and incidence of SRS tended to progress with time (50% at 5 months after SE and 87.5% at 7 months after SE). Rats that experienced SE at P25 were monitored at 5 months after SE and seizures were detected in 83.3% of animals. Cell count of hippocampal neurons performed after video-EEG monitoring revealed loss of hilar neurons in both age groups. In P12 rats, morphological damage also tended to progress over time (Kubova and Mares, 2013). This recent study suggests that the animals that become epileptic after having undergone SE, at a time when the brain is immature, have been previously underestimated.

### Double-hit injury models

In addition to the model of SE, different types of injury and/or prolonged seizures have been used to induce

**Table 1.** Summary of the animal models for prolonged seizures in the immature brain.

| | Mode of induction of model | Age | Cell injury after SE in the immature brain | Epileptogenesis/spontaneous recurrent seizure |
|---|---|---|---|---|
| SE<br>Lithium-pilocarpine | LiCl followed by pilocarpine | P7-Adult | P7: Very little<br>P14: CA1<br>P21: Hilus-CA3 | No<br>Yes in 10-20%<br>Yes in almost 100% |
| Hyperthermic seizure | Heated airstream | P10-P11 | No | Yes<br>35% after 26 min HS<br>45% after 64 min HS |
| Hypoxia | Unilateral carotid ligation | P7 | Yes | Yes 56 %<br>100% when cerebral infarcts<br>0% in absence of brain injury |
| Hypoxia | Global hypoxia for 15 min using an airtight chamber | P10 | No | Yes |

HS: hyperthermic seizure; P7: seventh post-natal day; SE: status epilepticus.

epileptogenesis, with the goal of mimicking human conditions (*table 1*). Hyperthermic seizures induced in postnatal-day-10 or -11 (P10-P11) rat pups are used to mimic prolonged febrile seizures (Toth *et al*., 1998). Hypoxic-ischaemic brain insults can also be induced in rat pups to model hypoxic-ischaemic encephalopathy, which is a prominent cause of mortality in neonates and morbidities, including epilepsy, in children. Animal models involving hypoxia in P7 to P10 rats have been used to study both acute neonatal seizures and the subsequent development of epilepsy (Kadam *et al*., 2010; Rakhade *et al*., 2011).

The use of models with a double hit provides data that allows us to further understand the contribution of early-life events in the future development of epilepsy. The motivation for developing "two-hit" animal models was to include a clinical relevant component to prolonged early-life seizures. As an example, a prolonged febrile seizure often precedes the development of temporal lobe epilepsy with mesial temporal sclerosis (Cendes, 2004). In order to evaluate the role of inflammation as a component of febrile seizures in children (Auvin and Vallee, 2009), we recently conducted a study using a double-hit injury approach (SE+systemic inflammation). We have reported that inflammation increases SE-induced cell injury at both P7 and P14 (Auvin *et al*., 2007). The combination of systemic inflammation to P14 SE results in an increase of epileptogenesis (Auvin *et al*., 2010). Similarly, the assessment of the role of focal cortical dysplasia in the occurrence of prolonged seizures in the developing brain is possible in some experimental studies. Using rat pups that had a right sensorimotor cortex freeze lesion at P1, followed by hyperthermic seizure at P10, the rats with a localised freeze lesion had a lower seizure threshold and experienced prolonged ictal manifestations suggesting that focal cortical lesion is involved in atypical febrile seizures (Scantlebury *et al*., 2004). Moreover, this pre-existing cortical lesion modified epileptogenesis following a prolonged seizure. The same experimental group reported that 86% of patients with the lesion plus the hyperthermic seizure group experienced development of SRS, while none of the controls exhibited any abnormality (Scantlebury *et al*., 2005).

### Effect of anticonvulsant treatment and anti-epileptogenesis treatment

The use of antiepileptic drugs to stop SE in humans has been evaluated in the model of SE. The cessation of SE by diazepam decreases the level of SE-induced cell injury (Pitkanen *et al*., 2005; Francois *et al*., 2006). The use of 2.5 mg/kg of diazepam in adult rats stops seizures and decreases the mortality rate. In addition, the cessation of seizures by diazepam results in a lower level of cell loss in CA-1, in the hilus, and in the piriform cortex, compared to controls (Francois *et al*., 2006). When SE is stopped by diazepam (20 mg/kg) 2 hours or 3 hours after SE, the outcome is modified. The rate of animals that develop epilepsy after SE was reduced when diazepam was given to stop SE. The percentage of epileptic animals was lower (42%) in the 2-hour DZP group, compared to the 3-hour DZP group (71%). Moreover, the seizures were less frequent in the DZP-treated group, with a marked reduction when treatment was given after 2 hours of SE (Pitkanen *et al*., 2005).

Despite vast laboratory evidence that many anticonvulsant medications possess neuroprotective properties (Pitkanen, 2002; White, 2002), pharmacological strategies to mitigate or prevent epileptogenesis in humans have failed (mostly in head trauma) (Holtkamp and Meierkord, 2007; Loscher and Brandt, 2010). This failure might be related to several issues such as species differences, inappropriate timing of intervention, focus on the wrong molecular targets, differential genetic susceptibility, and perhaps long-term neurotoxicity of drugs (Pitkanen and Lukasiuk, 2011). It has been suggested that the molecular targets that have traditionally been studied may be more relevant to ictogenesis (*i.e*. induction of an acute seizure) rather than epileptogenesis (Kobow *et al*., 2012).

### Conclusion

Animal models of SE have provided a better understanding of the diversity of behavioural, structural, and functional changes resulting from SE. It is now well established from the experimental studies that the consequences of SE are mainly related, but not entirely, to the developmental age when SE occurs. Regarding the criteria of animal models (see above), a good model of the acute stage of SE is provided. However, it should be stated that none of the models using initial injury to induce epileptogenesis can be considered as models of paediatric epilepsy, because the occurrence of SRS during adulthood is always reported (Auvin *et al*., 2012).

It is obvious that animal studies have also inherent limitations; SE is induced by the administration of noxious agents and is not spontaneous. The experimental data show that the outcome appears to be species- or model-specific. It is currently difficult to draw any definitive statements regarding model specificity because the data described in the literature are often confounded by differences in the route of administration of the drug, strain of rat used, and duration of SE.

Despite these limitations, animal models of SE support the notion that SE induces brain damage. The younger the age at the time of SE, the less severe the consequences appear to be. However, several factors, including the cause of SE (evaluated by double-hit injury models), modify the seizure severity as well as the level of injury and related epileptogenesis.
The use of treatment to stop the seizure and/or the duration of the SE results in a decrease of SE-induced cell injury. Preliminary data suggest that anti-epileptogenesis treatment with a single target is limited, while multiple targets should be further explored in order to reach the realistic goal of an anti-epileptogenesis treatment (Kobow *et al*., 2012). □

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# Overview of clinical efficacy and risk data of benzodiazepines for prolonged seizures

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**ABSTRACT** – An historical overview is provided regarding the use of benzodiazepines for the treatment of acute prolonged convulsive seizures. It is clear that intravenous benzodiazepines remain a first step for the in-hospital treatment of prolonged seizures or status epilepticus. However, in the community, in a pre-hospital situation, intravenous administration is not possible. In recent years, it was shown that rectal, buccal, intranasal, and intramuscular administration of benzodiazepines is very effective as a first and safe treatment step. In many cases, rectal diazepam is not socially acceptable anymore, and therefore more emphasis is now put on buccal, intranasal, and intramuscular administration. At present, based on the available data, midazolam is the product of choice for the acute treatment of prolonged convulsive seizures.

**Key words:** epilepsy, acute prolonged seizures, benzodiazepine, midazolam, diazepam, status epilepticus

The risks of acute and especially prolonged convulsive seizures are now well established. Apart from possible secondary injuries (*e.g.* head trauma, drowning, and burning), prolonged seizures can induce significant medical emergencies such as cardiac arrhythmias, lung congestion, liver failure, and rhabdomyolysis (Jovic *et al.*, 2011; Kravljanac *et al.*, 2011; Varelas *et al.*, 2013). Increased seizure duration is associated with increased morbidity. The effects of prolonged seizures on the epileptogenic process is still a matter of debate, but preliminary data from the FEBSTAT study indicate that a prolonged febrile seizure can entail MRI-visible changes in about 10% of the children scanned within 72 hours of their seizure (see also Van Landingham *et al.* [1998]). Some of these children will go on to develop mesial temporal sclerosis and concurrent refractory temporal lobe epilepsy (Shinnar *et al.*, 2012; Harden, 2013; Yoong *et al.*, 2013). By definition, a prolonged convulsive seizure is the start of an imminent status epilepticus and appropriate action to stop the seizure is therefore warranted. It is reassuring to see

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that many doctors now include an acute seizure treatment protocol in their overall epilepsy treatment plan (Lagae, 2011; Wait *et al*., 2013). Until recently, acute treatment was still reserved for a medical setting, such as an ambulance or the emergency department. Since a critical turning point for developing status epilepticus is at around 5-10 minutes of seizure activity, initial action in a medical setting might already be too late (Shinnar *et al*., 2001). Smith *et al*. (1996) showed that 80% of the seizures lasting for more than five minutes will continue for >30 minutes. Therefore, attention has shifted towards "pre-hospital" and "community-based" treatment options. This implies that a first-treatment action could also be administered by non-medically trained people, such as parents, teachers, or a partner of the patient. It is very clear from the community-based study of Chin *et al*. (2008) that pre-hospital treatment significantly reduces the duration of status epilepticus.

This first step in the treatment of a convulsing patient should therefore be effective, simple, and safe. In this review, we will therefore focus on the benzodiazepines (BZP), as these are the most potent and direct-acting drugs to stop an ongoing seizure. In particular, we will discuss the non-intravenous use of BZP, as these are the possible drug formulations for treatment in pre-medical community settings. The mechanism of BZP is well known; BZP activate the GABA-A receptor, thereby inducing a hyperpolarisation with a decrease of excitability (for a detailed review on BZP mechanism and GABA receptors, see, for instance, Galanopoulou [2008] and Sankar [2012]).

## The gold standard: intravenous benzodiazepines

The study of Alldredge *et al*. (2001) is a pivotal study that illustrates the efficacy of intravenous BZP in status epilepticus. In this study, 258 adults with a status epilepticus were randomly assigned to three treatment groups: lorazepam (2 mg), diazepam (DZP) (5 mg), or placebo. This pre-hospital treatment was given by trained ambulance personnel. In about 30% of the two active study groups, a second similar dose of the study drug was given. This was similarly performed for 50% of the placebo group. One of the primary outcome parameters was the cessation of the status epilepticus upon arrival at the emergency department of the hospital. In both active treatment groups (lorazepam and DZP), the mean duration of the prolonged seizure was around 30 minutes (lorazepam: 34±17.8 minutes; DZP: 31.3±14.5 minutes; placebo 46.7±38.8 minutes), indicating that indeed the majority of patients suffered from a status epilepticus, if one accepts the 30-minute-duration definition of status epilepticus. More than 50% of all patients were known to have epilepsy and one of the most frequent causes (in overall around 20%) of their status epilepticus was a chronic antiepileptic drug level in the blood that was too low, probably illustrating the well known, but underestimated, low treatment compliance in epilepsy. In the three groups, it took around 16 minutes to get to the emergency department. In the lorazepam and DZP groups, the status epilepticus was terminated in 59.1 and 42.6%, respectively. In the placebo group, spontaneous cessation was observed in only 21.1%. Although this difference between active treatment and placebo is very clear, one should not ignore that a large group continued to have a status epilepticus even after BZP treatment. One of the most likely explanations is the delayed administration of adequate rescue drugs, again a strong argument to start treatment in the community, before the arrival of medically trained personnel. Further analysis showed that lorazepam was significantly more effective than DZP. This finding represented the start to promote intravenous lorazepam as the first step in the majority of treatment protocols of status epilepticus. The study also demonstrates the high morbidity of status epilepticus; 47.8% (DZP group), 56.9% (LZP group), and 63% (placebo group) of patients had to be admitted to intensive care for further diagnosis and treatment. Neurological deficits at hospital discharge were observed in around 16%. Perhaps even more illustrative were the mortality data; in the LZP and DZP groups, this was 7.7 and 4.5%, respectively, but as high as 15.7% in the placebo group.

## Influential non-intravenous BZP studies

Probably, the first study using rectal DZP for the treatment of acute seizures was published in 1979 by Knudsen (1979). Rectal DZP (<3 years: 5-7.5 mg; >3 years: 7.5-10 mg) was given to 44 children with 59 seizure episodes. Children were between six months and five years and all seizures were generalised convulsive seizures. Of the 59 seizures, 35 were febrile seizures. With regards to febrile and non-febrile seizures, the results were the same, and 80% of the treated seizures (43/54) stopped within 5 minutes. Another 7/54 of the seizures stopped after subsequent intravenous administration of DZP. A post hoc analysis showed that earlier administration of rectal DZP was associated with a higher chance of success; if given before 15 minutes of seizure duration, seizures were stopped in 96%. Later treatment (>15 minutes) was successful in 57%, which is indeed comparable to the data reported in the Alldredge study. In the Knudsen study, the rectal DZP administration did not cause any significant respiratory depression.

Respiratory depression clearly is the most important possible side effect to deal with, but, as was already shown in this DZP study, it should not be overemphasized. In this respect, the study of Chin *et al*. (2008) clearly showed that respiratory depression can be observed only after two or more dosages of BZP. This was also confirmed in a recent study by Spatola *et al*. (2013). Therefore, when necessary, it is advocated to give a second dosage of BZP, but under medical supervision and best in a setting where respiratory support can be given.

The study of Dreifuss *et al*. (1998), albeit using a different study design and outcome parameters, basically confirms the Knudsen study. In the Dreifuss study, feasibility of rectal DZP gel for prolonged seizures in a home-based setting was studied. Rectal DZP (0.5 mg/kg for children 2 to 5 years of age, 0.3 mg/kg for children 6 to 11 years of age, and 0.2 mg/kg for patients 12 or older) was compared to placebo. One of the efficacy outcome parameters was the recurrence of a seizure within an observation period following administration (12 hours for children). In the DZP group, 62% remained seizure-free in the next 12 hours, whereas this was the case in only 24% in the placebo group. The time to first seizure recurrence was significantly longer in the DZP group than in the placebo group. Concerning side effects, respiratory depression was not reported in the patients receiving DZP. Only somnolence was observed more frequently in the DZP group (33%) than in the placebo group (11%). The fact that somnolence was also observed in the placebo group illustrates that this is a possible and well-known post-ictal phenomenon, and that not all sleepiness or somnolence should be attributed to BZP treatment. This Dreifuss study was very influential, and rectal DZP has since become the non-intravenous standard against which all other BZP preparations are compared.

Rectal administration of medication is not always easy and is no longer socially acceptable, especially for older children or adults. Other routes of administration and other BZP were therefore examined. Scott *et al*. (1999) compared buccal midazolam (MDZ) (at a dosage of 10 mg; 40 seizures in 14 patients) with rectal DZP (at a dosage of 10 mg; 39 seizures in 14 patients). All patients were children or adolescents, older than five years, presenting with prolonged seizures. Basically, this study clearly showed equal efficacy for both drugs; 75% of the seizures treated with buccal MDZ and 59% of the seizures treated with rectal DZP were responsive to the treatment. The median time between drug administration and end of the seizures was somewhat shorter for buccal MDZ (median: 6 minutes; range: 4-10 minutes) than for rectal DZP (median: 8 minutes; range: 4-12 minutes), but this difference was statistically not significantly different ($p$=0.31).

The study of McIntyre *et al*. (2005) examined in more detail, and in a larger patient group, the efficacy of buccal MDZ *versus* rectal DZP; 109 prolonged seizure episodes were treated with buccal MDZ and 110 with rectal DZP. In this study, the time to stop the seizures was significantly shorter for buccal MDZ (median: 8 minutes; range: 5-20) than for rectal DZP (median: 15 minutes; range: 5-31 minutes; $p$=0.01). In the MDZ group, 65% of the seizures stopped within 10 minutes, whereas this was the case in 41% of the DZP group. This difference in efficacy is also reflected in another outcome parameter; whereas subsequent intravenous lorazepam was required in 33% of the MDZ group, it was required in 57% of the DZP group. In addition, this study also investigated how many seizures recurred within one hour after initial success, defined as seizure cessation within 10 minutes. Again, there was clear difference between the two groups; early recurrence was observed in 14% in the MDZ group and in 33% in the DZP group. In this study, respiratory depression was carefully monitored and was defined as a fall in oxygen saturation or decrease in respiratory effort, sufficient to require assisted breathing. The rate of respiratory depression was very similar in both groups; 5 and 6% in the MDZ and DZP group, respectively.

The largest study comparing buccal MDZ to rectal DZP was performed in Uganda (Mpimbaza *et al*., 2008) and included 330 patients, 50% assigned to rectal DZP and 50% to buccal MDZ. The dosage for both drugs was similar: 2.5 mg for 3-11 months of age, 5 mg for 1-4 years, 7.5 mg for 5-9 years, and 10 mg for 10-12 years. The direct cause for the prolonged seizure was malaria in more than 60% in both groups. Similar outcome parameters to those used in the McIntyre study were included; seizure cessation 10 minutes after drug administration. Overall, there were no significant differences between the two treatment arms, but buccal MDZ was more effective in the non-malaria group; seizures treated with buccal MDZ stopped within 10 minutes in 74% (36/49), whereas this was the case in only 45% in the rectal DZP group (26/59; $p$=0.02). Looking in more detail, the median time for cessation of the seizure was around 4.5 minutes in the overall population and was not different between the MDZ and DZP groups. However, as in the McIntyre study, recurrence within one hour after initial success of the drug was higher in the DZP group (17.5%) than in the MDZ group (8%; $p$=0.026). Recurrence rate within 24 hours after initial success was also investigated, and was in fact relatively high in both groups; 46.3 and 39.1% in the DZP and MDZ group, respectively. Recurrence occurred earlier in the DZP group (median: 1.81 hours) than in the MDZ group (median: 5.11 hours; $p$=0.001). Although this study indeed dealt with a rather unique population, it basically confirms the McIntyre study, with data showing a better overall efficacy of MDZ.

Talukdar and Chakrabarty (2009) compared the efficacy of buccal MDZ (*n*=60) to intravenous DZP (*n*=60). This study allows us to look at the time differences induced by the necessary handling and preparation of the medication. It is expected that preparation of an intravenous drug will take more time than that for a drug for buccal administration. Essentially, both treatments were overall equally effective; seizures were controlled in 93% in the intravenous DZP group and in 85% in the buccal MDZ group. *Post-hoc* analysis did show a significant difference when different convulsive seizure types were considered, and complex partial seizures reacted better to intravenous DZP than to buccal MDZ; 100% *versus* 63.6%, respectively ($p$=0.01). For generalised tonic-clonic seizures, no differences were found (efficacy was measured at around 90% for both). The treatment initiation time (the time to deliver the medication) was, as expected, significantly longer for the intravenous preparation than for the buccal preparation; it took about twice as long to get the intravenous preparation ready. On the other hand, and again not surprising, the time for the drug to take effect was much shorter in the intravenous DZP group; mean time of 1.1 minutes for the intravenous DZP group *versus* 1.6 minutes for the buccal MDZ group ($p<0.001$). Overall, however, the time from seizure start to cessation was shorter in the MDZ group (mean: 2.39 minutes *versus* 2.98 minutes; $p$=0.004), indicating that the shorter preparation time of the medication significantly contributed to the success rate. However, one should not over-emphasize these, albeit small, time differences. The authors did conclude that their common practice to use intravenous medication as a first-line approach could indeed be replaced by the much easier buccal administration, if intravenous access was not easily possible.

Another way to deliver MDZ is the intranasal route, and in some countries this way of delivering MDZ is clearly preferred. In 2010, Holsti *et al*. published a study with a classic design, comparing intranasal MDZ (0.2 mg/kg; *n*=50) to rectal DZP (0.3-0.5 mg/kg; *n*=42) (Holsti *et al*., 2010). In the intranasal MDZ group, there was a shorter median cessation time compared to the DZP group (1.3 minutes faster; $p$=0.09). Further analysis also showed that intranasal administration is safe, although more children in the intranasal group (6%) needed extra oxygen compared to the intrarectal group (2%). This difference was not statistically significant.

Lahat *et al*. (2000) published their small randomised study, comparing intranasal MDZ (0.2 mg/kg) to intravenous DZP (0.3 mg/kg). In this study, BZP were used to treat febrile seizures in young children. Overall, both medications were equally effective if one looked at seizure cessation at 10 minutes; 23 of 26 seizures responded to initial treatment with intranasal MDZ and 24 of 26 responded to intravenous DZP. None of the treated children had clinical signs of respiratory depression. As in the Talukdar study, it took longer for the intravenous formulation to work, only because of the time needed to prepare drug administration.

In 2012, the first results of the RAMPART study (Rapid Anticonvulsant Medication Prior to Arrival Trial) were published (Silbergleit *et al*., 2012); the largest study on the use of BZP for the treatment of pre-hospital status epilepticus. This study was performed in children and adults and compared intramuscular MDZ (10 mg if body weight >40 kg) with intravenous lorazepam (4 mg). With regards to intention to treat, 448 patients were listed in the MDZ group and 445 patients in the lorazepam group. For this study, an autoinjector device was designed for rapid intramuscular injection of MDZ. Looking at the aetiologies of long-lasting seizures, about 30% were due to non-compliance or discontinuation of the prescribed antiepileptic drugs, and therefore, perhaps theoretically, preventable.

Seizures were arrested without additional rescue medication at arrival at the hospital (=primary outcome) in 73.4% in the intramuscular MDZ group and 63.4% in the intravenous lorazepam group. Here also, detailed timing analysis of the different treatment steps was provided. Time to administration of active treatment was significantly lower in the intramuscular MDZ group than in the intravenous lorazepam group (1.2 *versus* 4.8 minutes). On the other hand, time from active treatment to cessation of seizures was significantly shorter in the intravenous lorazepam group (1.6 *versus* 3.3 minutes), but this did not make up for the much faster preparation of the drug; overall, time from opening the box to cessation of the convulsions was still shorter for the intramuscular MDZ group. However, importantly, the large majority of seizures stopped well before 10 minutes in both groups.

Safety was assessed as one of the secondary endpoints. In both groups, about 14% required endotracheal intubation within 30 minutes after arrival at the emergency room. Most likely, this reflects the fact that the seizures did not stop in all patients and that more intensive care and second/third-line agents were needed to stop the seizures in these patients. It is therefore difficult to understand in how many patients this ventilatory support was needed because of pure drug side effects. Recurrence of seizures within 24 hours, again, was not different between both groups; 11.4 and 10.6% in the MDZ and lorazepam group, respectively. This large study clearly shows that intramuscular administration of MDZ is also a valuable option for treating long-lasting seizures. A primary condition remains the availability of a well-designed autoinjector with adjustable dosages for infants, children, and adults.

In 2010, McMullan *et al*. published a meta-analysis comparing DZP to MDZ for the treatment of status epilepticus in children and young adults (McMullan *et al*., 2010). All routes of drug administration were considered (intravenous, intramuscular, intrarectal, and intranasal). This study was undertaken before the publication of the RAMPART study. It should be noted that the definition of status epilepticus was variable but included definitions such as convulsions of >5 or 10 minutes, or convulsions upon entering the emergency room. This study therefore focused more on the effect of medication for prolonged seizures than for "classic" status epilepticus (>30 minutes duration of seizures). At that time, only six publications fulfilled the criteria for an adequate meta-analysis. Taking into account the small number and heterogeneity of the studies (especially with regards to route of administration), this analysis nevertheless confirmed that MDZ was superior to DZP (the risk ratio for MDZ to be superior was 1.52 [95% CI: 1.27-1.82]). Moreover, DZP was not effective in 170/386 seizures, and MDZ was not effective in 112/388 seizures. Respiratory safety was also analysed. Both drugs were equally safe in that respect; respiratory depression occurred in 3/375 seizures treated with DZP and in 2/375 seizures treated with MDZ.

Although almost no studies have been performed with intravenous or rectal MDZ, there is also limited experience with other diazepines and especially lorazepam (for an overview see Anderson and Saneto [2012]). Because of the superior results with intravenous lorazepam, it is indeed logical also to consider this product for non-intravenous use.

In 2011, Arya *et al*. (2011) published the results of their study comparing intravenous lorazepam (70 children) *versus* intranasal lorazepam (71 children). Dosage for both routes of administration was 0.1 mg/kg (max: 4 mg). Randomisation occurred at hospital entrance, hence the duration of the ongoing seizures was very variable but comparable in both groups. Both routes of administration were equally effective; seizure termination occurred within 10 minutes in 80 and 83.1% in the intravenous and intranasal group, respectively.

Ahmad *et al*. (2006) also found intranasal lorazepam to be a valid alternative for the treatment of prolonged seizures. They compared intranasal lorazepam with intramuscular paraldehyde and reported seizure cessation within 10 minutes in 75% in the intranasal lorazepam group *versus* 61.3% in the intramuscular paraldehyde group. Furthermore, in the lorazepam group, fewer patients required additional anticonvulsant agents compared to the paraldehyde group. They also confirmed the cardio-respiratory safety in both treatment arms.

## Concluding remarks

This short review of the clinical use of BZP for the treatment of long-lasting convulsive seizures illustrates that we do have effective drugs to stop the ongoing seizures in the majority of cases. Since early treatment to prevent status epilepticus is mandatory, non- intravenous use should be advocated. Rectal administration is no longer preferred, mainly because of social reasons, but also because of overall lower efficacy. MDZ is nowadays the drug of choice, with buccal, intranasal, or intramuscular routes of administration. For the use of possible alternative drugs, further details can be found in the review of this supplement by Chin (2014). Because first-line treatment should also be given by non-medical specialists, any treatment option should be simple and easy to use, with minimum handling and without any calculations to determine dosage. Only then can we hope for an earlier and more effective treatment of prolonged convulsive seizures, with eventually less status epilepticus and co-morbidities. There is an ongoing debate about who should be selected for acute treatment. Should every patient with epilepsy be informed and instructed about acute treatment options or should this be reserved for only those epilepsy syndromes with a high likelihood of developing long-lasting seizures? This debate should not be used as an argument to avoid discussion of acute treatment options for the majority of epilepsy patients. □

**Disclosures.**
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# What are the best ways to deliver benzodiazepines in children/patients with prolonged convulsive seizures?

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**ABSTRACT** – Aetiology is the main determinant of morbidity and mortality in convulsive status epilepticus (CSE) but longer seizure durations may also increase risk of worse outcome. Thirty minutes of seizure activity is usually the time period used in longstanding definitions of CSE but it is not acceptable to wait for 30 minutes before treatment. Whilst intravenous therapy is best, pre-hospital treatment by a non-intravenous route is most practical in treating children. Benzodiazepines are the main class of first-line emergency antiepileptic drugs. This review will examine the available data on benzodiazepines according to: stability in the conditions of the emergency room services, drug absorption *via* non-intravenous route, clinical efficacy and safety, and ease of delivery and social acceptability.

**Key words:** benzodiazepine, children, seizure

Convulsive status epileptics (CSE) is associated with increased morbidity and mortality (Raspall-Chaure *et al*., 2006). Whilst underlying aetiology is the main determinant of morbidity and mortality, experimental and clinical data indicate that longer seizure durations also increase the risk of a worse outcome (Lothman, 1990). Thirty minutes of seizure activity is the time from which permanent brain injury can be observed and hence the reason why this time period is used in longstanding definitions of status epilepticus, particularly for epidemiological studies. However, it is not acceptable to wait for 30 minutes before treatment. Since 80% of seizures that do not stop within five minutes of onset continue for at least 30 minutes (Shinnar, 2007) and the longer seizures continue the more difficult they are to stop, there has been the emergence of an operational definition of CSE for treatment purposes to be based around a 5-minute threshold (Lowenstein *et al*., 1999). Since most seizures start outside of the hospital/clinical setting (Raspall-Chaure *et al*., 2007), it is imperative that in order to minimise seizure duration, treatment should be started prior to arrival at the hospital (Treiman *et al*., 1998; Chin *et al*., 2004a; Chin *et al*., 2008).

Whilst there is clear evidence that intravenous therapy is best (Yoong *et al*., 2009), pre-hospital treatment

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by a non-intravenous route is most desirable since intravenous access poses a major challenge in a child experiencing seizures, particularly in children under the age of 5 years, when CSE is most common (Chin *et al*., 2004b; Chin *et al*., 2006). Benzodiazepines are the main class of first-line antiepileptic drugs (AEDs) used for the emergency treatment of seizures because they have a rapid onset of action, have good efficacy, and are easy to prepare and administer by non- intravenous routes.

The first licensed non-intravenous benzodiazepine preparation was rectal diazepam, and for many years was considered the gold standard for pre-hospital treatment (Shorvon, 2013). However, new benzodiazepines have emerged (midazolam, clonazepam, and lorazepam) with alternative delivery routes (intranasal, buccal/oromucosal, sublingual, and intramuscular). This review will examine the available data according to:

– stability in the conditions of the emergency room services (EMS);
– rapid drug absorption via non-intravenous route ;
– proven clinical efficacy and safety;
– ease of delivery route and social acceptability.

## Stability in the conditions of the EMS

Rectal diazepam gel is stable under the temperature and light exposure conditions found in ambulances. When exposed to a freeze-thaw cycle, hard freeze (-30°C for 72 hours), extreme light exposure, and long-term evaluation at either 30°C or 40°C, diazepam gel concentration always exceeded 95% of label, with no substantial changes in excipients or physicochemical properties (Alldredge *et al*., 2002).

Similarly, when exposed to ambient ambulance conditions during the spring/summer months in 14 metropolitan areas across the United States, midazolam and lorazepam were generally stable. There was no degradation in a non-commercially available parenteral, liquid, autoinjector preparation of midazolam after 60 days (mean relative concentration: 1.00; 95% confidence interval [CI]: 1.00-1.00); it was stable across temperature exposures (adjusted R2: -0.008). Under the same conditions, there was minimal degradation of a commercially-available, parenteral, liquid preparation of lorazepam (mean relative concentration: 0.99; 95% CI: 0.98-0.99), but degradation correlated to increasing mean kinetic temperature (adjusted R2: 0.278). Whilst there was a statistically significant difference ($p<0.001$) in the temperature dependence degradation between both drugs, both maintained clinically acceptable concentration levels of the active drug (McMullan *et al*., 2013). McMullan and colleagues have now confirmed that these findings remain consistent even when storage under EMS conditions is increased to 120 days (McMullan; *personal communication*). These findings are important since they provide evidence against the long held view that parenteral, liquid lorazepam requires refrigerated storage, and therefore justify the possibility of its usage in the pre-hospital setting.

## Rapid drug absorption and action *via* non-intravenous route

*Table 1* summarises the bioavailability, time to maximum blood concentration ($T_{max}$), and plasma half-life ($T_{1/2}$) of buccal midazolam, intranasal midazolam, sublingual lorazepam, sublingual clonazepam drops, and rectal diazepam (Wermeling *et al*., 2006; Riss *et al*., 2008; Viropharma, 2011). Generally, there is high bioavailability amongst all, with greatest bioavailability of 90% or more for sublingual FDDF lorazepam and rectal diazepam. Whilst $T_{max}$ for buccal and intranasal midazolam, as well as rectal diazepam, is within 30 minutes (the shortest being 10 minutes for intranasal midazolam), that of clonazepam drops and sublingual FDDF lorazepam is longer, between 1-4 hours.

**Table 1.** Bioavailability, time to maximum blood concentration ($T_{max}$ ), and plasma half life ($T_{1/2}$) of buccal midazolam, intranasal midazolam, sublingual lorazepam, sublingual clonazepam drops, and rectal diazepam.

| | **Oromucosal midazolam hydrochloride** (Viropharma, 2011) | **Intranasal midazolam** (Wermeling *et al*., 2006) | **Clonazepam drops** (Tassinari, 1998; Greenblatt, 1982) | **Fast-dissolving drug formulation Lorazepam** (Riss *et al*., 2008) | **Rectal diazepam** (Riss *et al*., 2008) |
|---|---|---|---|---|---|
| Bioavailability | 75%†– 87%‡ | 72.5% | >80% | 94% | 90% |
| $T_{max}$ | <30 min* | 10.3 min | 1–4 hours | 2.3 hours | 10–45 min |
| $T_{1/2}$ | 27 min-3.4 hours | 3.25 hours | 19-60 hours | 7-26 hours | 21-70 hours |

*In children; †In healthy adults; ‡In children with severe malaria and convulsions.

For both children and adults, there is some evidence of inter- and intra-subject variability with regards to the effective dose and speed of onset of sublingual lorazepam (Greenblatt *et al*., 1982; Camu *et al*., 1988; Yager and Seshia, 1988). In a study of 10 children, 8 had a latency of 30-60 minutes in terminating serial seizures (Yager and Seshia, 1988). In a randomised, single, five-way crossover study comparing the pharmacokinetics of two forms of sublingual lorazepam compared to intravenous, intramuscular, and oral lorazepam, 10 healthy adult volunteers each received a modest dose of 2 mg of lorazepam administered by each route, with sequential phlebotomy to assess levels. Of those given standard oral tablets of lorazepam administered sublingually, there was a lag time of 22.7±5.1 minutes from administration to the start of absorption in nine of the 10 volunteers. When they were given a special tablet preparation delivered sublingually, 8 showed delayed absorption of 14.9±3.5 minutes. There was no lag time for the intravenous nor intramuscular delivery routes. All subjects reported mild to moderate discomfort when given the drug intramuscularly. All routes exhibited similar peak drug concentrations, with the pharmacokinetics of the sublingual routes being similar to that of the oral route, which in turn, had a slower time-to-peak concentration compared to intramuscular lorazepam, although this was not statistically significant (Greenblatt *et al*., 1982). This study suggests that if a preparation of lorazepam that undergoes rapid dissolution is available, it may be a useful clinical alternative to intramuscular/intravenous lorazepam. Fast-dissolving drug formulation (FDDF) lorazepam may be one such preparation but its absorption can be variable.

In a study comparing the pharmacokinetics of FDDF and intravenous lorazepam as anaesthetic premedication, 8 adult surgical patients were given 4 mg sublinguales FDDF lorazepam and 8 were given 4 mg intravenous lorazepam. The absorption of sublingual FDDF lorazepam was rapid in half of the patients and provided a high plasma concentration of lorazepam (Cmax: 61.8±16.2 ng/mL) in a short time interval (Tmax: 58±39 min). However, in the other half, the absorption was slow with a low plasma concentration (Cmax: 39.5±17.2 ng/mL) with a variable lag time (Tmax: 360±69 min). This variability in absorption might explain why premedication with FDDF lorazepam is sometimes less effective than expected (Camu *et al*., 1988), and could theoretically result in similar problems in the acute treatment of seizures.

In a study on the pharmacokinetics of buccal midazolam, 10 healthy adult volunteers were given 10 mg of buccal/sublingual midazolam hydrochloride. There was a rapid increase in venous blood concentrations for the first 20-30 minutes following treatment. However, preceding the venous spike, spectral analysis identified EEG changes at 8- to 30-Hz frequencies, 5-10 minutes into the test, but not in control subjects (Scott *et al*., 1998). These data prove that there is a rapid cerebral effect of midazolam hydrochloride and this occurs before a detectable venous surge. This may be attributable to the highly lipophilic, closed-ring form that midazolam hydrochloride assumes when it becomes absorbed into the circulation (Scott *et al*., 1998). To the author's knowledge, there is, to date, no peer-reviewed similar published data on buccal midazolam maleate.

## Proven clinical efficacy and safety

The duration of action of rectal diazepam is less than 2 hours, for buccal midazolam hydrochloride is 3 to 4 hours, and is longer for clonazepam drops (24 hours), and even longer for sublingual FDDF lorazepam (up to 72 hours). Duration of action is not correlated with the plasma concentration-time profiles of these drugs (Rey *et al*., 1999). In principle, the longer duration of action of midazolam, clonazepam, and lorazepam may favour the use of these as alternatives to diazepam, particularly if they are available in a suitable non-intravenous rapidly absorbable form. In support of this, a metanalysis of six studies comparing non-intravenous midazolam and rectal or intravenous diazepam showed that midazolam was superior to diazepam in achieving seizure cessation (risk ratio: 1.52; 95% CI: 1.27-1.82). However, there are few randomised controlled trials that compare the different benzodiazepines and different administration routes for efficacy and safety in the emergency treatment of seizures in children. Those available are summarised below.

### Intranasal midazolam *versus* rectal diazepam

In a prospective randomised study comparing intranasal midazolam *versus* rectal diazepam for the home treatment of acute seizures in paediatric patients with epilepsy, 358 children who attended a paediatric neurology clinic were prescribed home emergency treatment for their next seizure (Holsti *et al*., 2010). Carers were randomised to use either 0.2 mg/kg of intranasal midazolam up to a maximum of 10 mg or 0.3 to 0.5 mg/kg of rectal diazepam, up to a maximum of 20 mg RD (maximum, 20 mg) for their child's seizure at home if it lasted more than five minutes. Ninety-two carers gave emergency treatment and completed the study; 50 administered intranasal midazolam and 42 gave rectal diazepam. Four died

during the study without having used emergency medication, 254 did not receive emergency medication during the study, and 8 withdrew from the study. There was no statistically significant difference in time to treatment administration, time from treatment to seizure cessation, or efficacy between intranasal midazolam and rectal diazepam. Using an 11-point nominal scale to investigate ease of administration and overall carer satisfaction, carer scores were higher with intranasal midazolam compared to rectal diazepam for both ease of administration (10 *vs*. 9; $p$=0.02) and satisfaction (9.3 *vs*. 7.3; $p$=0.02). Four children (8%) treated with intranasal midazolam required oxygen or intubation for respiratory complications, compared to one (2%) treated with rectal diazepam (the difference was not statistically significant).

### Intranasal midazolam *versus* intravenous diazepam

*Table 2* summarises the results of two studies that have compared intranasal midazolam hydrochloride to intravenous diazepam (Lahat *et al*., 2000; Mahmoudian and Zadeh, 2004). In both, the end point was seizure termination within 10 minutes of treatment. The study by Lahat and colleagues was restricted to febrile seizures whilst Mahmoudian and Zaden included febrile and afebrile seizures. Both studies found remarkably high seizure termination rates when either drug was used. In particular, the study by Mahmoudian and colleagues reported seizure termination within 10 minutes of treatment in 100% of patients treated with either intranasal midazolam or intravenous diazepam. This is much higher than that found in any other study examining the efficacy of either drug for the emergency treatment of seizures.

Both studies found that buccal midazolam was quicker to administer than intravenous diazepam. However, Mahmoudian and Zadeh found that the interval between treatment and seizure termination was longer with those treated with buccal midazolam (3.6 minutes) compared to those given intravenous diazepam (2.9 minutes) ($p$=0.007).

Increased blood flow to the nasal mucosa during an upper respiratory tract infection may aid drug absorption but nasal secretions may theoretically dilute the midazolam solution, thereby reducing contact with the absorptive nasal mucosal surface (Lahat *et al*., 2000). In the study by Lahat and colleagues, most of the 26 children treated with intranasal midazolam had an upper respiratory tract infection, of which only 3 had poor seizure control which was attributable to presumed ineffective absorption, however, there were

**Table 2.** Randomised controlled trials of intranasal midazolam hydrochloride *versus* intravenous diazepam for the emergency treatment of seizures.

| | **Lahat *et al*., 2000** | | **Mahmoudian and Zadeh, 2004** | |
|---|---|---|---|---|
| No. patients | 47 | | 70 | |
| Type of seizures | Febrile only | | Febrile and Afebrile | |
| Age range | 0.5-5 years | | 0.17-15 | |
| Treatment[a] | Nasal Midazolam 0.2 mg/kg | IV Diazepam 0.3 mg/kg | Nasal Midazolam 0.2 mg/kg | IV Diazepam 0.2 mg/kg |
| No. of episodes (no. of patients) | 26 | 26 | 35 | 35 |
| Seizure termination within 5-10 mins (%) | 23 (88) | 24 (92) | 35 (100) | 35(100) |
| $p$ for difference in proportions | (NS) | | (NS) | |
| Time to administer[c]/min (range) | 3.5 (0-7) | 5.5 (1.5-9.5) | Not analysed but reported to be shorter for nasal midazolam compared to intravenous diazepam | Not analysed but reported to be shorter for nasal midazolam compared to intravenous diazepam |
| Time to response[c] /min | 6.1 | 8.0 | 3.6 | 2.9 |

NS: significant; IV: intravenous.

no confirmatory drug levels. Nasal irritation was not investigated in either of the trials on intranasal midazolam described above (Lahat *et al*., 2000; Mahmoudian and Zadeh, 2004; Holsti *et al*., 2010). However, there is a potential for irritation to the nares, including burning sensation and lacrimation attributable to the acidic pH (3.5) of midazolam solution (Lugo *et al*., 1993).

### Buccal midazolam *versus* rectal diazepam

*Table 3* summarises studies comparing buccal midazolam *versus* rectal diazepam for emergency seizure treatment (Scott *et al*., 1999; McIntyre *et al*., 2005; Mpimbaza *et al*., 2008; Ashrafi *et al*., 2010). Except for the study by Ashrafi and colleagues in which buccal midazolam maleate was used, all the studies used buccal midazolam hydrochloride. In all the studies, the primary endpoint was clinical cessation of motor activity within 5-10 minutes of administration. Together, these data provide strong evidence that buccal midazolam is superior or, at the very least, as effective as rectal diazepam. Amongst these studies, one was carried out in a residential school for children with severe epilepsy, whilst the others were carried out in the accident and emergency setting where participants are likely to have difficult-to-control seizures because of the length of seizure activity prior to arrival at hospital. Thus, although there are no trials based on the general population, it would be reasonable to surmise from the available data that buccal midazolam would be better for out-of-hospital treatment. The results of the Ugandan study (Mpimbaza *et al*., 2008), which arguably had the best design since it was blinded and had the largest sample size, show that buccal midazolam was superior to diazepam in children without malaria but not in those with malaria. These findings highlight aetiology as playing a substantial role in effectiveness of treatment. In all the studies, respiratory depression was similar or less frequent with treatment with buccal midazolam, compared to treatment with rectal diazepam.

### Buccal midazolam *versus* intravenous diazepam

A hospital-based study of 120 children (82 males; age range: 0-12 years) compared seizure termination within five minutes after treatment with 0.2 mg/kg of buccal midazolam *versus* 0.3 mg/kg of intravenous diazepam, irrespective of seizure duration or cause (Talukdar and Chakrabarty, 2009). Of the children treated with buccal midazolam, 85% had seizure termination within five minutes of treatment, compared to 93% in those treated with intravenous diazepam ($p$=0.142). Although treatment with buccal midazolam was initiated quicker than that with intravenous diazepam, the time required to control seizures was shorter in those treated with intravenous diazepam. For up to 10 minutes post-treatment, no patients in either group had unusual CNS depression, respiratory depression, apnoea, or cardiac dysrhythmia. These data highlight that intravenous treatment is better, but buccal midazolam would be a reasonable option if intravenous access proves problematic.

### Intramuscular midazolam *versus* intravenous diazepam

Chamberlain and colleagues carried out a prospective, randomised study comparing intramuscular midazolam with intravenous diazepam for the treatment of seizures with a duration of at least 10 minutes in 24 children (Chamberlain *et al*., 1997). The 13 children in the midazolam group received medication earlier (3.3±2.0 *vs*. 7.8±3.2 minutes, respectively; $p$=0.001) and their seizures terminated earlier (7.8±4.1 *vs*. 11.2±3.6, respectively; $p$=0.047) than those of the 11 children treated with diazepam.

### Intramuscular midazolam *versus* intravenous lorazepam

The Rapid Anticonvulsant Medication Prior to Arrival Trial (RAMPART) study was a double-blind, randomised, non-inferiority trial of the effectiveness of intramuscular midazolam *versus* intravenous lorazepam in seizure management in the out-of-hospital setting for all age groups (Silbergleit *et al*., 2012). In total, 145 paediatric patients among 893, overall, were enrolled. For those who were seizure-free upon arrival at hospital, there was no statistical difference among those treated with intramuscular midazolam (73%) *versus* treatment with intravenous lorazepam (63%), however, the rate of hospitalisation was lower in the intramuscular midazolam group (57.6 *vs*. 65.6%, respectively; relative risk: 0.88). This study highlights the potential for another non-intravenous out-of-hospital route of administration. However, the authors admitted that the cost of study autoinjectors, that were not commercially available and had been funded through the USA's Department of Defense, would have been prohibitively expensive for general usage. Respiratory depression (14%) and hypotension (1-2%) were comparable in both treatment groups.

### Intramuscular diazepam *versus* placebo

Abou-Khalil *et al*. carried out a double-blind, randomised, placebo-controlled trial of a diazepam autoinjector administered by caregivers to patients with epilepsy who required treatment for acute

**Table 3.** Studies comparing buccal midazolam *versus* rectal diazepam for emergency seizure treatment.

| | **Scott *et al.*, 1999** | | **McIntyre *et al.*, 2005** | | **Mpimbaza *et al.*, 2008** | | **Ashrafi *et al.*, 2010** | |
|---|---|---|---|---|---|---|---|---|
| Blinding | None | | None | | Single-blinded | | None | |
| Setting | High-income, Western European country. | | High-income, Western European country. | | Low-income, African country | | Middle-high income, Middle Eastern country | |
| Sites | Single | | Multi-centre | | Single | | Two | |
| Pre-hospital/ Hospital | Pre-hospital. Residential school for young people with severe epilepsy. | | Hospital (emergency department of 3 children's hospitals and one general hospital) | | Hospital (paediatric emergency department of referral centre) | | Hospital (emergency department of 2 paediatric referral centres) | |
| Aetiology | Convulsive seizures in a patient with epilepsy | | Convulsive seizures of any aetiology | | Convulsive seizures of any aetiology. Most malaria-related | | Convulsive seizures of any aetiology | |
| No. patients | 28 | | 177 | | 330 | | 98 | |
| Age range | 5-19 years | | 1-6 years | | 3 m-12 years | | Not given but median age 24 months in midazolam group and 48 months in the rectal diazepam group | |
| Treatment | Mid 10 mg | Dia 10 mg | Mid 0.5 mg/kg | $Dia_r$ 0.5 mg/kg | Mid 0.5 mg/kg | Dia 0.5 mg/kg | Mid 0.3-0.5 mg/kg | Dia 0.5 mg/kg |
| No. of episodes (no. of patients) | 40 (14) | 39 (14) | 109 (92) | 110 (85) | 165 (165) | 165 (165) | 49 (49) | 49 (49) |
| Seizure termination within 5-10 mins (%) | 30 (75) | 23 (59) | 61 (56) | 30 (27) | 115 (70) | 94 (57) | 49 (100) | 40 (82) |
| *p* for difference in proportions | 0.16 | | <0.0001 | | 0.016 | | <0.001 | |
| Mean or Median Time to administer/min (range) | 2 (1-4) | 2 (1-3) | - | - | - | - | 2 | 3 |
| Mean or MedianTime to response /min | 6.00 | 8.00 | 8.00 | 15.00 | 4.75 | 4.35 | 4 | 5 |
| Recurrence >1 hour post treatment/% | - | - | 14 | 33 | 39 | 46 | - | - |
| Time to recurrence/ hours (IQR) | - | - | <1 | <1 | 5.1 (1.1-10) | 1.8 (0.9-3.5) | - | - |

repetitive seizures (Abou-Khalil, 2013). Among the 234 subjects enrolled, 81/110 were given placebo and 82/124 given diazepam. Those given intramuscular diazepam had a longer time to next seizure with a hazard ratio of 0.55 (95% CI: 0.34-0.88; $p$=0.012) and a lower number of seizures experienced during the 12-hour post-dose period (median: 0.0), compared to placebo (median: 1.0; $p$=0.010). Injection site pain was similarly common in both groups (15% for placebo *vs*. 17% for diazepam), as was injection site haemorrhage (6% for placebo *vs*. 5% for diazepam).

Whilst there are no randomised trials for lorazpam or clonazepam delivered sublingually, buccally or intranasally, there is anecdotal evidence of their effectiveness by these routes in the acute treatment of seizures.

Midazolam delivered buccally or intranasally has a relatively short half-life of up to 3.4 hours, compared to the other benzodiazepines included in *table 1* which may have a half-life of a day or longer. Long elimination half-life increases the risk of a hangover effect. In the randomised trials of intranasal/buccal midazolam *vs*. rectal diazepam described above, the risk of respiratory depression was similar or less in children treated with midazolam, compared to rectal diazepam.

## Ease of delivery route and social acceptability

There is overwhelming evidence of a preference for nasal/oromucosal midazolam over rectal diazepam by families, carers, and affected children themselves (Wilson *et al*., 2004; Terry *et al*., 2007; Sofou *et al*., 2009). The preference is mainly due to better personal dignity for those being treated, the fact that buccal/intranasal routes are more socially appropriate, ease of administration for wheelchair users, and a perceived quicker response relative to rectal diazepam. A US 2003-2004 survey found that 19% (8/43) of parents reported that schools had refused to administer rectal diazepam, citing legal concerns behind their refusal (Terry *et al*., 2007). A recent European review revealed that fear of liability was a critical issue for treatment with rectal diazepam by non-parental carers (Wait *et al*., 2013).

Early in the placebo-controlled trial of a diazepam autoinjector, the study was put on hold because three caregivers accidentally injected themselves while attempting to administer study medication to subjects. Thus, in addition to the common complications of pain at the site and haemorrhage in patients given intramuscular treatments, there is the potential for needle stick/injection injuries to care-givers.

## Conclusion

Midazolam, clonazepam and lorazepam are all in principle viable options to diazepam for the treatment of prolonged seizures in children. For practical reasons, non-intravenous delivery options must be the mainstay of early emergency treatment of children with seizures out of hospital, and include treatment rectally, intranasally, buccally (*via* oromucosa), sublingually (*via* oromucosa), or intramuscularly. The evidence, thus far, is that buccal midazolam is superior to rectal diazepam, but beyond that, there are limited conclusions that can be made because of the limited randomised controlled trials comparing benzodiazepines delivered by different routes.

There is marked diffidence towards treatment by the rectal route, and alternatives are at least as effective in seizure termination. There is rapid absorption by the nasal route but treatment by this route can theoretically be associated with marked nasal irritation, although this would be less of an issue in an acute convulsing child. There are also concerns surrounding this route of delivery because of a theoretical reduced absorption in children with upper respiratory tract infections, but this has to be counterbalanced by increased blood flow that may aid absorption. These theoretical concerns do not seem to have been borne out in practice, as a randomised controlled trial restricted to children with febrile seizures, many of whom had upper respiratory tracts, reported a high proportion of children successfully treated with nasal midazolam. However, why aim for a smaller moving target in the nares of children to deliver an intranasal preparation when there is a much bigger and easily accessible option close by for delivery of a buccal preparation? There is a theoretical risk of aspiration with buccal treatment but none of the studies on buccal midazolam above have reported this as a complication. Intramuscular injections cause local haemorrhage and are painful which would be a major deterrent for use in children, especially in those who require recurrent usage.

It is evident from the studies included in this review that the optimum dose for the individual drugs, according to the varied delivery route options, remains unclear. The long-term effects of these medications and whether they have a neuroprotective effect beyond seizure termination is not known. Further studies in these areas, particularly randomised controlled trials, are needed. □

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Prolonged Epileptic Seizures:
identification and treatment

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# Developmental pharmacology of benzodiazepines under normal and pathological conditions

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**ABSTRACT** – Benzodiazepines are allosteric agonists of $GABA_A$ receptors ($GABA_AR$), pentameric ligand-gated $Cl^-$ channels, which serve both an important neurodevelopmental role but are also the principal inhibitory system in the brain. However, their subunit composition, channel properties, and function, as well as their region-specific expression patterns, change through development. These processes have been extensively studied in rodents and to some extent confirmed in higher species. Specifically, $GABA_AR$s acquire faster kinetics with age and their pharmacology changes rendering them more sensitive to drugs that have higher affinity for $\alpha$1 subunit-containing $GABA_AR$s, such as benzodiazepines, but also, their inhibitory function becomes more potent as they shift from having depolarising to hyperpolarising responses due to a shift in $Cl^-$ gradient and cation chloride cotransporter expression. Concerns have been raised about possible pro-apoptotic and paradoxical effects of benzodiazepines in the neonatal normal rat brain, although it is unclear, as yet, whether this extends to brains exposed to seizures. Growing evidence indicates that the pharmacology and physiology of $GABA_AR$s may be altered in the brain of rats or humans with seizures or epilepsy, or different aetiologies that predispose to epilepsy. These changes follow different paths, depending on sex, age, region, cell type, aetiology, or time-point specific factors. Identification of dynamic biomarkers that could enable these changes *in vivo* to be monitored would greatly facilitate the selection of more effective agonists with fewer side effects.

**Key words:** $GABA_AR$ subunit, epilepsy, chloride cotransporter, hyperthermia

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Benzodiazepines are allosteric agonists of $GABA_A$ receptors ($GABA_AR$s), pentameric ligand-activated Cl- channels that typically mediate $Cl^-$ inflow leading to neuronal hyperpolarisation and fast inhibitory postsynaptic currents. Benzodiazepines act in the presence of GABA, and their effects greatly depend upon the type of subunits present in the $GABA_AR$s. Their affinity is greatest for $GABA_AR$s containing $\alpha$1 and $\gamma$2 subunits. The inhibitory effects of benzodiazepines in combination with their availability as formulations that

permit rapid and flexible delivery (*e.g.* buccal, nasal, rectal), even in situations when intravenous access is not available, have established them as the first-line rescue drugs or treatment for rapid cessation of ongoing seizures throughout life.

However, the effects of $GABA_AR$ agonists may change under certain normal or pathological situations, in which either the subunit composition is not optimal or the function of $GABA_ARs$ is altered. Here, we will review the animal studies that have highlighted the developmental changes in $GABA_AR$ physiology and pharmacology of their agonists, including benzodiazepines, and discuss these findings with regards to their potential relevance for the clinical use of benzodiazepines in the treatment of ongoing seizures, and especially in very young individuals.

## $GABA_AR$ structure and pharmacology

There are currently 16 known subunits in mammals (six α, three β, three γ, and one δ, ε, θ, and π). Each $GABA_AR$ consists typically of five subunits, with the most commonly observed arrangement being a combination of two α, two β, and one γ subunit (*figure 1*). Subunit composition dictates a number of receptor characteristics, including localisation within each cell or in various brain regions, affinity for ligands and drugs, as well as regulation by specific signalling pathways. For example, typically (but not always), $GABA_ARs$ that contain γ subunits are located post-synaptically, and their activation is recorded as phasic inhibitory postsynaptic currents (IPSCs) (Mody and Pearce, 2004; Farrant and Nusser, 2005; Mohler, 2006a). However, substitution of a γ for a δ subunit causes extrasynaptic localisation and results in tonic $GABA_AR$ activation by ambient GABA molecules (tonic currents) (Nusser *et al*., 1998; Farrant and Nusser, 2005). Specific subunit composition is not only responsible for subcellular localisation, but also impacts the kinetics of the receptors, as well as their affinity for certain ligands (Mohler, 2006a). As an example, α3 subunits located in extrasynaptic receptors have been shown to desensitize much more slowly than α2 subunits located in synaptic receptors (Devor *et al*., 2001).

Whereas the GABA binding site lies between an α and a β subunit, the benzodiazepine binding pocket is between an α and a γ subunit, and their affinity is greatest for $GABA_ARs$ which contain α1 and γ2 subunits, intermediate for α2 or α3-containing $GABA_ARs$, low for δ-containing $GABA_ARs$, and absent for those that contain α4 or α6 subunits. Consequently, the primary target of benzodiazepines is the potentiation of postsynaptic $GABA_ARs$, that are already activated by synaptically released GABA quanta, and which mediate phasic GABA inhibition.

Since specific subunit composition has such far-reaching effects on kinetics and pharmacology, the differential expression of $GABA_AR$ subunits across brain regions, cell types, between genders, and as the brain develops, adds to the diversity of their final effects (Mohler, 2006a; Mohler, 2006b). The regional differences in subunit expression are also thought to contribute to the different functional effects of agonists that prefer certain subunit combinations (Mohler, 2006a). For example, α1 is found more in the thalamus, cortex, brainstem, hypothalamus, and hippocampus, and agonists may have antiseizure, sedative, and amnesic effects. In contrast, selective agonists for α2 and α3-containing $GABA_ARs$ exhibit anxiolytic or myorelaxant effects. However, most of these studies are largely on adult populations and as we will discuss later, the effects in the developing brain may be quite different.

## $GABA_AR$ structure and pharmacology change through development

During normal development, the affinity of $GABA_ARs$ for agonists or antagonists changes due to age-, region- and sex-specific patterns of $GABA_AR$ subunit expression, subcellular localisation, or mRNA editing. Most of these studies have been performed in rodents. The significantly shorter lifespan of rodents (approximately two years) warns that brain development in rodents occurs relatively faster than in humans. In general, a postnatal day (PN) 8-13 rat or mouse is considered to be equivalent to a human newborn full-term baby, based on gross measures of brain growth (weight, protein/DNA content). However, unlike human babies, eye opening only occurs between PN13-15 in rodents. Weaning from the dam usually takes place at around PN21 and puberty starts between PN32-35, whereas adulthood begins on PN60. In fact, when comparing rodent to human development, each developmental process may mature at its own tempo and cross-species extrapolation may not be very accurate.

$GABA_AR$ subunit expression changes through development following cell-type and region-specific patterns. Although such developmental changes are widespread, there is considerable diversity across regions and cell types. However, a well-documented "subunit switch" between the α2/α3 and α1 subunits has attracted a lot of interest as it has been observed in multiple studies, regions, and species, and may potentially account for important age-related differences in $GABA_AR$ inhibitory responses and their pharmacology. Laurie *et al*. described increasing α1 subunit expression with age in the telecenphalic cortex, diencephalon, thalamus, hippocampus, and cerebellum (Laurie *et al*., 1992). At the same time, a

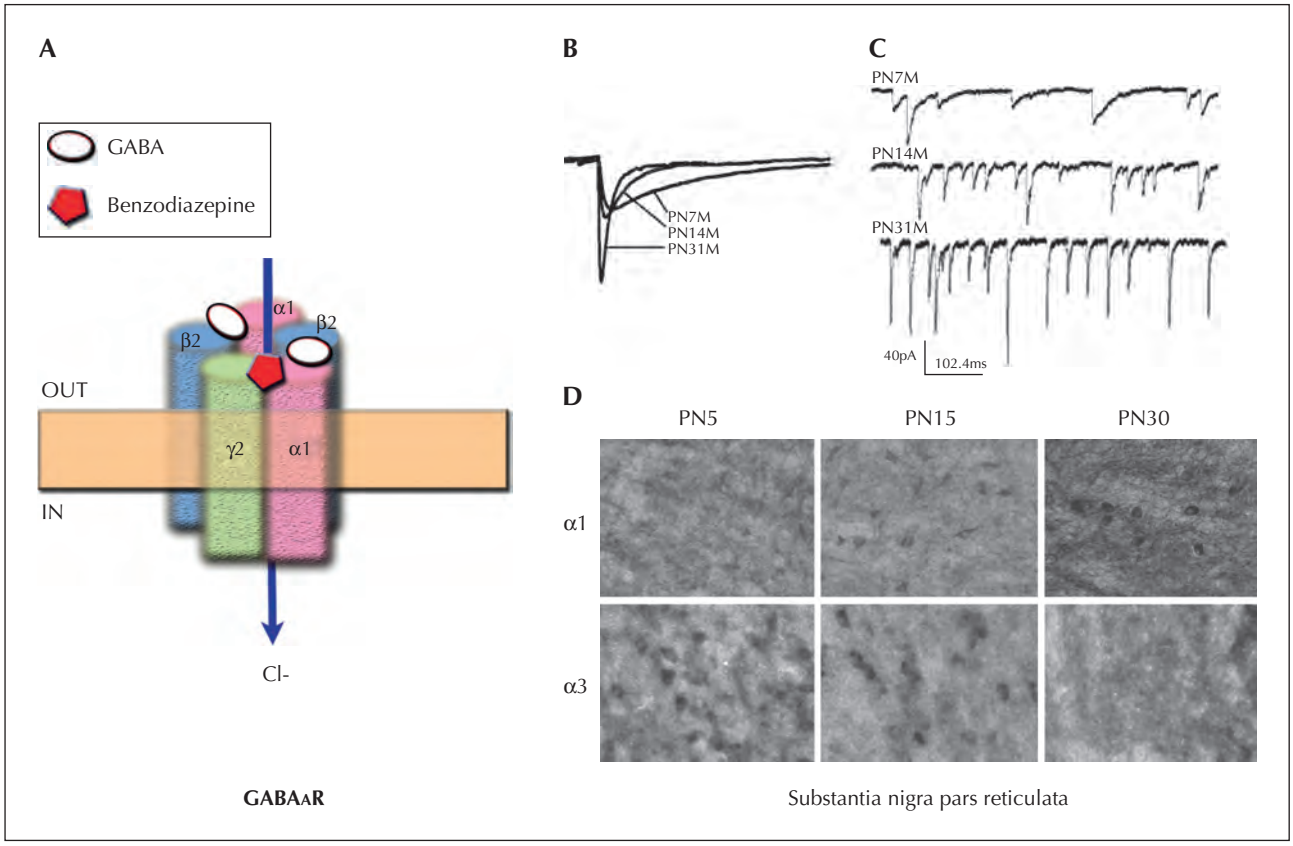


**Figure 1.** Developmental changes in $GABA_AR$ composition and postsynaptic currents. (A) $GABA_ARs$ are pentameric ligand-gated $Cl^-$ channels that are typically comprised of 2α and 2β subunits, in addition to a fifth subunit which is usually a γ subunit, although other subunit combinations have been described. The GABA binding pocket lies between the α and β subunits, whereas the benzodiazepine binding site is between the α and γ subunits, with the α1γ2 combination exhibiting greatest affinity. (B-D) During development, a gradual shift in the subunit composition of $GABA_ARs$ has been described in several brain regions. Whole-cell patch clamp recordings from GABAergic SNR neurons are shown here to demonstrate that in older age groups, the kinetics of post-synaptic $GABA_AR$ inhibitory currents acquire faster kinetics (faster rise and decay times) (B) and become more frequent (C). This can be explained by the gradual replacement of α3 subunits (highly present in immature neurons) and α1 subunits (highly expressed in mature neurons), as shown in the substantia nigra neurons (D), by performing immunochemistry specific to either α1 or α3 subunits.
Permission to reproduce panels B-D from Chudomel *et al*. (2009) was obtained by Elsevier.

simultaneous decline in α3 (telencephalon, cortex, diencephalon, thalamus, and cerebellum) and, in certain regions, α2 (diencephalon, thalamus) occurs. Similar observations have also been documented in the basolateral amygdala (Ehrlich *et al*., 2013) and substantia nigra pars reticulata (SNR) (Chudomel *et al*., 2009) (*figure 1*). The replacement of α2/α3 subunits by α1 alters not only the kinetics of phasic $GABA_AR$ inhibitory postsynaptic currents (IPSCs), rendering them faster, but also decreases the affinity of these receptors to drugs such as zolpidem or benzodiazepines. For example, in the rat SNR, PN5-9 SNR GABAergic neurons had low frequency and slow $GABA_AR$-IPSCs, whereas in peripubertal PN28-32 rats, IPSCs were recorded with high frequencies and rapid kinetics and were more sensitive to zolpidem, a preferential agonist of α1 (Chudomel *et al*., 2009). A similar switch has also been documented in the monkey prefrontal cortex, with high levels of α2 mRNA in one-week-old monkeys and a gradual replacement by high α1 mRNA levels in adult monkeys (Hashimoto *et al*., 2009). Studies in humans have been limited to post-mortem tissues, for obvious ethical reasons. Two independent studies in the human cerebral cortex reported increasing expression of α1 and γ2 mRNA between the neonatal and adult ages, while the level of mRNA of other subunits, such as α2 (Fillman *et al*., 2010) or α4 (Jansen *et al*., 2010), declined.

Another point of developmental regulation is at the mRNA editing level, which may change the function of the translated proteins. In the case of α3 subunit, the level of RNA editing leading to an isoleucine-to-methionine substitution in the region coding the third transmembrane domain was very low during mouse embryonic development, reached maximal levels at PN7, and was observed in 90% of adult mouse

brains (Rula *et al*., 2008). The edited and non-edited forms of α3 subunit confer distinct channel properties. $GABA_A$Rs containing non-edited α3 activate rapidly and deactivate more slowly than those that contain edited α3 subunits, but also exhibit stronger outward rectification (*i.e*. $Cl^-$ influx). This particular feature renders the non-edited α3 subunit-containing $GABA_A$Rs more capable of mediating shunt inhibition in the face of depolarising currents, and can therefore be protective early in development, when $GABA_A$R currents can be depolarising, as will be discussed in the following section.

## Changes in depolarising to hyperpolarising $GABA_A$R signalling with age

The direction of $GABA_A$R responses depends upon the Cl- gradient between the intra- and extracellular space and also, to a degree, on $HCO3^-$. The intracellular $Cl^-$ concentration is controlled by cation-$Cl^-$ cotransporters, of which the most widely studied is the Cl- importer $Na^+/K^+/Cl^-$ cotransporter 1 (NKCC1) and the $Cl^-$ exporter $K^+/Cl^+$ cotransporter (KCC2) (*figure 2*). Early in life, in most studied neurons, there is a relatively high intracellular $Cl^-$ concentration, due to a relative abundance of NKCC1 over KCC2. As a result, opening of the $GABA_A$R mediates $Cl^-$ efflux, leading to depolarisation. The early immature depolarising effects of $GABA_A$ receptors are important for development; nevertheless, they lead to calcium processes necessary for neuronal processes including cell migration and differentiation (Ben-Ari, 2002; Galanopoulou, 2008a). Precocious deprivation of depolarising GABA from developing neurons may lead to dysmature features, such as underdeveloped arborization of neuronal processes in cortical neurons (Cancedda *et al*., 2007; Wang and Kriegstein, 2008; Wang and Kriegstein, 2011). It should be noted that these normal depolarising effects of GABA are not necessarily excitatory, since they may not always trigger action potentials. Furthermore, in the setting of excessive excitation, as during seizure activity, $GABA_A$R agonists may still exert inhibitory effects, through shunting inhibition, although this type of inhibition is less effective (Staley and Mody, 1992).

During development there is a gradual shift, from an NKCC1 dominant state, early on, to a KCC2 dominant state in more mature neurons. This eventually reduces the intracellular $Cl^-$, resetting the $Cl^-$ electrochemical reversal potential, and resulting in $Cl^-$ influx and hyperpolarisation after $GABA_A$R activation (Plotkin *et al*., 1997; Rivera *et al*., 1999) (*figure 2*). The timing of shift from depolarising to hyperpolarising $GABA_A$R signalling varies across brain regions, neuronal types, and between sexes, adding another level of complexity to the understanding of GABAergic signalling. Such examples are the substantia nigra pars reticulata (SNR) and CA1 pyramidal neurons of the hippocampus of Sprague-Dawley rats, where $GABA_A$R activation elicits sex-specific effects, with delayed disappearance of depolarising responses in males compared to females (Galanopoulou *et al*., 2003; Kyrozis *et al*., 2006; Galanopoulou, 2008b). Neurons in the SNR of male rats were shown to start manifesting hyperpolarising $GABA_A$R responses by postnatal day (PN) 17, while in females the switch occurred much earlier, at PN10 (Kyrozis *et al*., 2006), consistent with increased KCC2 mRNA in the SNR of female rats at all ages (Galanopoulou *et al*., 2003). This sexually dimorphic pattern continues across other brain regions, including CA1 pyramidal neurons. Again, female rats showed isoelectric or mildly hyperpolarised $GABA_A$R as early as PN4, while males started to have hyperpolarising responses around PN14 (Galanopoulou, 2008b). Systemic administration of $GABA_A$R agonists have also demonstrated sex-specific effects in intracellular processes, such as phosphorylation of the cAMP responsive element binding protein (pCREB), which are probably linked to the depolarisation-induced calcium rises (Auger *et al*., 2001; Galanopoulou *et al*., 2003; Perrot-Sinal *et al*., 2003; Galanopoulou, 2006; Mantelas *et al*., 2007; Galanopoulou, 2008c). Sex differences in KCC2 and/or NKCC1 have been proposed to underlie the earlier onset of hyperpolarising $GABA_A$R signalling in females (Galanopoulou *et al*., 2003; Galanopoulou, 2008b; Damborsky and Winzer-Serhan, 2012; Murguia-Castillo *et al*., 2013). KCC2 expression is also under the control of gonadal hormones in immature rat SNR neurons. According to a proposed hypothesis, the high endogenous levels of oestrogenic derivatives of testosterone aromatization in the newborn male rat brain may actually delay the developmental rise of KCC2 and the appearance of hyperpolarising GABA in certain brain regions, such as the SNR (Galanopoulou, 2008c). In humans, the only evidence corroborating the idea that there may be depolarising GABA in very immature neurons comes from post-mortem studies, where high NKCC1 and low KCC2 expression were shown in cortical extracts from neonates, followed by a rapid decline in NKCC1 and gradual increase in KCC2 during the first few months of life (Dzhala *et al*., 2005; Jansen *et al*., 2010).

## $GABA_A$R agonists have age-specific effects

What is the impact of all these structural and functional changes of $GABA_A$Rs upon the effects of drugs

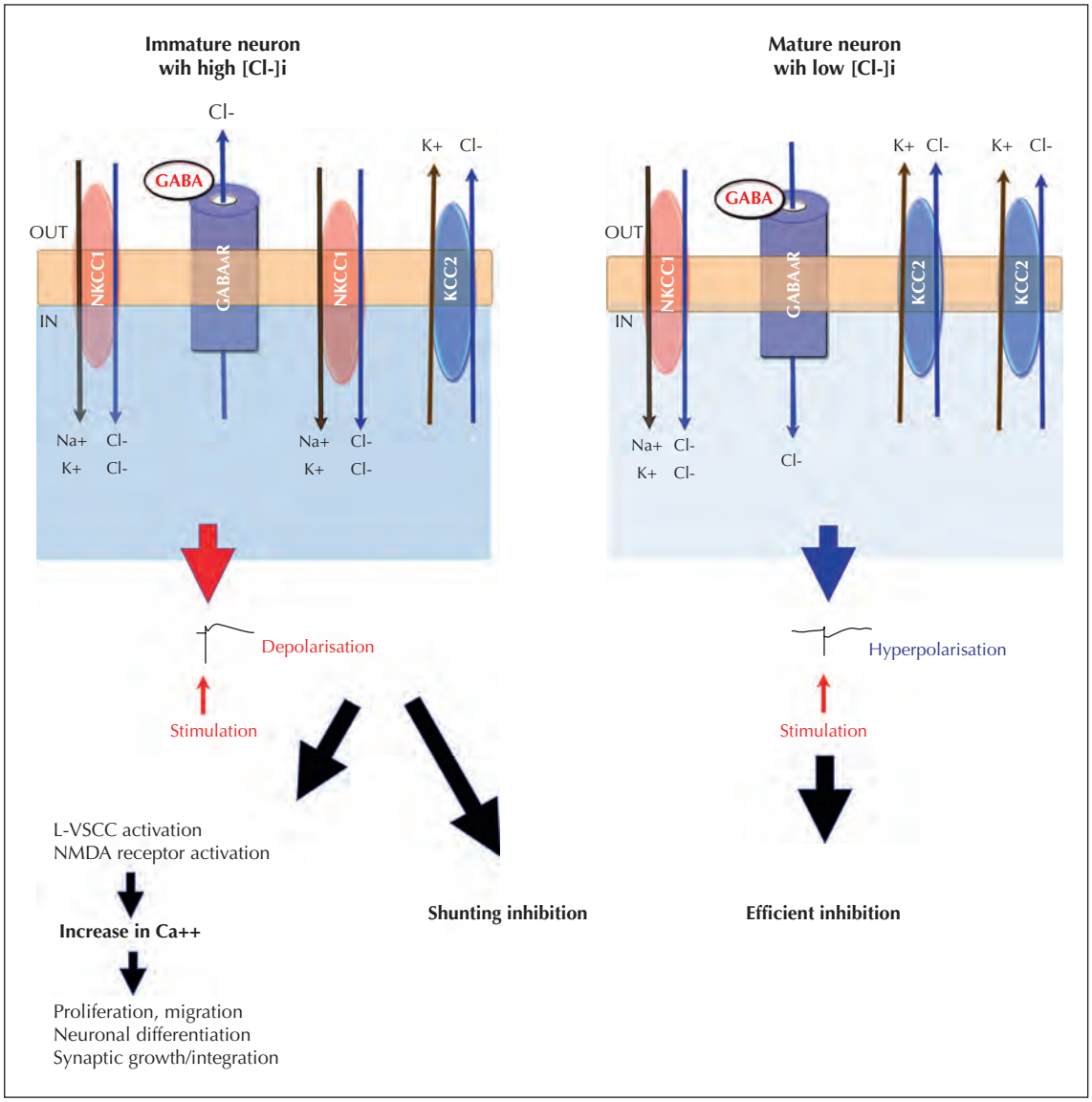


**Figure 2.** Developmental changes in the physiology of $GABA_A$Rs. $GABA_A$R can normally elicit depolarising or hyperpolarising post-synaptic responses depending upon the electrochemical gradient of $Cl^-$, between the intra- and extra-cellular space. In immature neurons, there are abundant cation chloride cotransporters that import $Cl^-$ (*e.g.* NKCC1) and scarce cation chloride cotransporters that export $Cl^-$ (*e.g.* KCC2), creating relatively high intracellular $Cl^-$ concentrations ($[Cl^-]_i$). As a result, opening of $GABA_A$Rs leads to efflux of $Cl^-$, depolarising these neurons. GABA depolarisations are necessary early in life as they support calcium-sensitive processes that are important for neuronal proliferation, migration, differentiation, and synaptic growth and integration. GABA depolarisations are not necessarily excitatory, as they do not necessarily trigger action potentials. In the face of excessive neuronal excitation (*e.g.* during seizures), the open $GABA_A$R channels can still shunt excitatory currents, contributing a weak form of inhibition (shunting inhibition). In mature neurons, there is greater activity of KCC2 than NKCC1, which lowers $[Cl^-]$i and permits the hyperpolarising $GABA_A$R currents to emerge, once $GABA_A$Rs open. This suggests that the efficacy of inhibitory effects of benzodiazepines may be enhanced in older age groups.

such as benzodiazepines or barbiturates through development? Animal studies have addressed this question in normal animals and have supported the idea of age-specific effects of GABAergic drugs. Increased apoptotic death and reduced neurogenesis is observed in one-week-old rats exposed to benzodiazepines or phenobarbital (Bittigau *et al.*, 2002; Bittigau *et al.*, 2003; Stefovska *et al.*, 2008). Similar findings were observed with other antiseizure drugs, suggesting that the immature brain may be more dependent upon a higher level of neuronal activity than the adult for survival. Midazolam is reported to decrease the mechanical reflex threshold and increase the magnitude of mechanical and thermal reflexes in

neonatal rats, but had no effect in juvenile rats (Koch *et al*., 2008). In the same study, midazolam had sedative effects in PN10 and PN21 rats but not in PN3 rats. Although it is tempting to associate these paradoxical effects of midazolam in newborn rodents with the early depolarising effects of $GABA_AR$ signalling, the answer is not always as simple. The final output of the inhibition or activation of a neuronal cluster in the brain is largely dependent on the way a neuronal network is organised, interconnected, and functionally active. For example, infusions of the $GABA_AR$ agonist muscimol in the SNR, which although ultimately silences local neuronal activity, may produce anticonvulsant or proconvulsant effects, depending on the age of the rat, whether it is male or female, and whether it is infused in the anterior or posterior SNR (Veliskova and Moshé, 2001; Veliskova and Moshé, 2006).

Even more importantly, drugs are typically given to target a specific disease process or symptom and their effects cannot be extrapolated to those observed in a diseased brain. For example, although benzodiazepines may potentially accentuate cell death in newborn rats, these studies were performed in normal rodents and not in animals that exhibit status epilepticus, which, on its own merit, may have adverse developmental outcomes. Therefore, careful evaluation, including evaluation of long-term outcomes, is warranted when assessing the efficacy and tolerability of these drugs in very young subjects.

## Seizures and epilepsy can disrupt $GABA_AR$ signalling

Multiple studies have investigated the association between either induced seizures, the epileptic state or different aetiology and the expression and function of $GABA_AR$ subunits (reviewed in Galanopoulou and Moshé [2014]). Using the kainic acid or the pilocarpine (with or without lithium) models as methods to test the effects of status epilepticus on the expression of $GABA_AR$ subunits, there is no singular pattern of changes that emerges. Rather, the effects of seizures depend upon the age at seizure induction, the time-point when subunit expression is studied, and subunit-specific, but also model and region-specific, factors (*figure 3*). For example, the expression of $\alpha$1 subunit is increased in the dentate gyrus of adult rats exposed to early-life pilocarpine-induced status epilepticus (Zhang *et al*., 2004; Raol *et al*., 2006), regardless of whether these were epileptic or not, but reduced in those exposed to pilocarpine status epilepticus in adulthood (Brooks-Kayal *et al*., 1998). Expression studies of $GABA_AR$ subunits in post-surgical specimens from epilepsy patients with focal cortical dysplasias also suggest a disrupted developmental expression profile of the $GABA_AR$ subunits which is also dependent upon the type of dysplasia (Jansen *et al*., 2010).

A study that has initiated a lot of interest into the potential pathogenic role of depolarising $GABA_AR$ signalling in epilepsy reported an aberrant presence of depolarising $GABA_AR$ signalling in human epileptic subiculum and paradoxical silencing of interictal epileptic discharges by $GABA_AR$ inhibitors (Cohen *et al*., 2002). This was attributed to reduction in KCC2 expression (Huberfeld *et al*., 2007). Multiple reports confirmed similar observations in temporal lobe epilepsy (Palma *et al*., 2006; Munoz *et al*., 2007) or other pathological epileptic conditions, including hypothalamic hamartomas (Kim *et al*., 2009), cortical dysplasias (Aronica *et al*., 2007; Jansen *et al*., 2010; Shimizu-Okabe *et al*., 2011; Talos *et al*., 2012), and tumour-associated epilepsy (Conti *et al*., 2011). However, based on the human studies, it is not possible to differentiate between the contribution of epilepsy, aetiology, treatments, stressors or other factors on the observed changes in $GABA_AR$ signalling. In animal studies, where some of these factors can be better controlled, pathological reversal to depolarising $GABA_AR$ signalling with concomitant increase in NKCC1 or decrease in KCC2 have been documented in selected brain regions of adult rodents subjected to status epilepticus (Benini and Avoli, 2006; de Guzman *et al*., 2006; Pathak *et al*., 2007; Bragin *et al*., 2009). On the other hand, the response of immature rats to seizures is again quite different and may be dependent upon sex-specific factors (Galanopoulou, 2008b) or the time after seizure occurrence (Galanopoulou, 2008b; Khirug *et al*., 2010). For example, kainic acid status epilepticus in neonatal male rats with depolarising $GABA_AR$ signalling in the hippocampus causes a premature appearance of hyperpolarising $GABA_AR$ signalling, unlike that observed in adult rats (Galanopoulou, 2008b). It is currently, however, unclear whether this is a protective mechanism or if it contributes to the developmental deficits that ensue.

The biggest question therefore is how to use all this knowledge in a manner that will translate into more effective and safer use of GABAergic drugs in humans, particularly in very young individuals, with seizures or epilepsy. Even if we were to account for all the variables involved (age, sex, region, aetiology, cell type, etc.), the expression and function of $GABA_ARs$ may change with time or in response to external stressors or internal comorbid or physiological factors that will require a live and dynamic predictive modelling method. The discovery and implementation of dynamic, safe biomarkers with sufficient temporospatial readout detail will be critical.

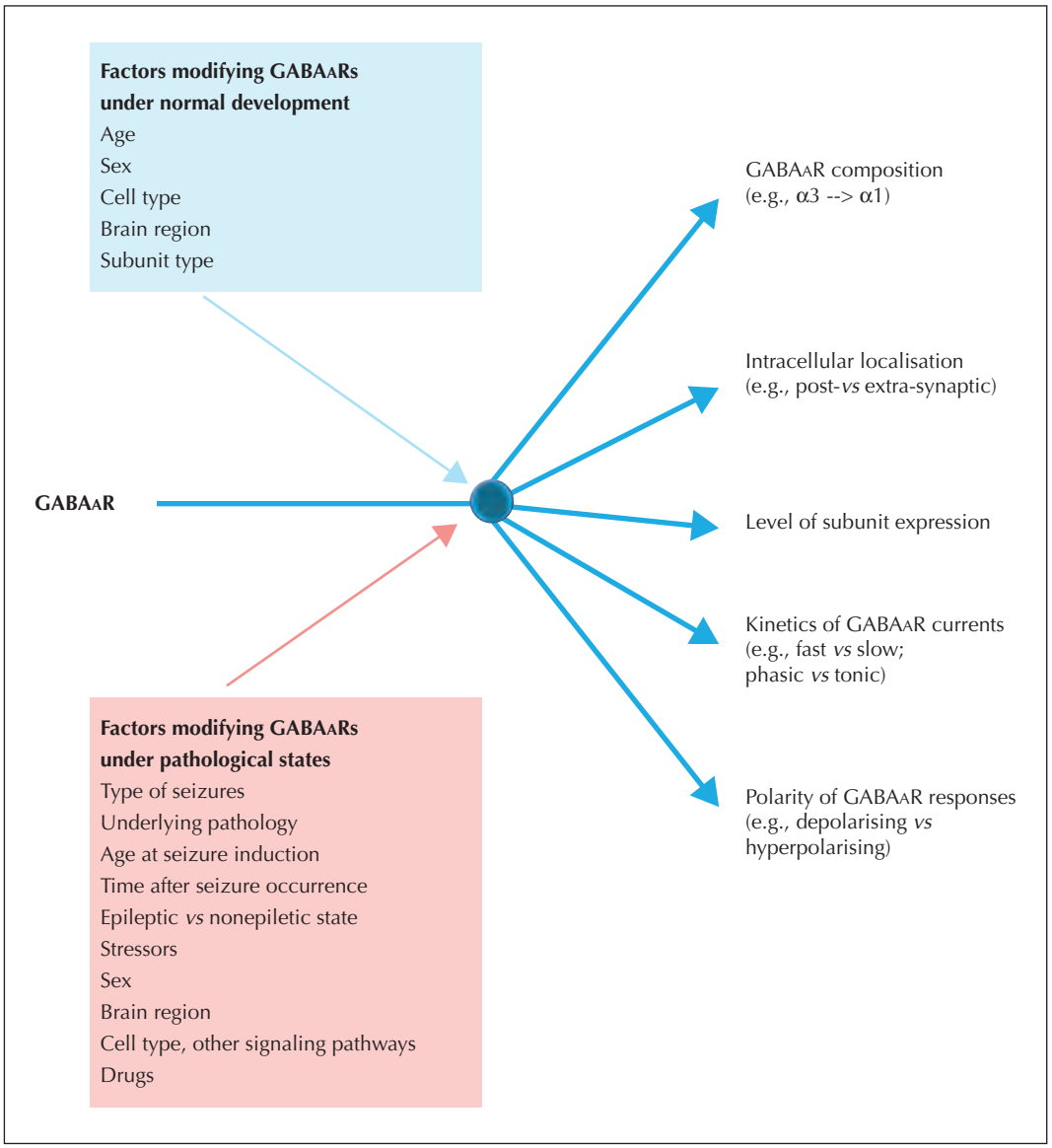


**Figure 3.** $GABA_AR$ expression and functional changes during normal development and pathological states. Various factors contribute to the variable functions of $GABA_ARs$ and strength of $GABA_AR$ inhibition under normal conditions, including age, sex, cell type, brain region, and subunit types expressed. In addition, seizures, underlying pathological states (aetiology), age at seizure induction, time after seizures, the presence of epileptic state, and other stressors or drugs may further diversify the function of $GABA_ARs$. As a result, the end effect and efficacy of benzodiazepines may vary across brain regions, subjects, and under different normal or pathological states.

## Hyperthermic seizures may alter $GABA_AR$ signalling

Of particular interest is the role of fever on $GABA_AR$ signalling, given the high prevalence of febrile seizures in the paediatric population. Several studies have looked specifically at the role of hyperthermia with variable observations. Hyperthermia can impair $GABA_AR$ signalling by decreasing $GABA_AR$ IPSCs, increasing GABA uptake, reducing selective α $GABA_AR$ subunits, or cause retention of $GABA_AR$ subunits in the endoplasmic reticulum (Fujii *et al.*, 2001; Kang *et al.*, 2006; Sharma, 2006; Swijsen *et al.*, 2012). On the other hand, hyperthermia may enhance $GABA_AR$ signalling in selective neuronal populations or increase benzodiazepine-sensitive $GABA_ARs$ in certain brain regions (Gonzalez Ramirez *et al.*, 2007; Swijsen *et al.*, 2012). An interesting study has also proposed that reversal to depolarising $GABA_AR$ signalling after hyperthermic seizures induces ectopic granule cells that may contribute to adult epilepsy (Koyama *et al.*, 2012). It should be noted, however, that the role of aberrant

seizure-induced neoneurogenesis in epileptogenesis has not been confirmed in other models (Sankar *et al*., 2000).

## Conclusions

In summary, the specific differences in expression and function of $GABA_ARs$ related to age, anatomical region, sex, receptor subunit, and disease state make it clear that great care should be taken when it comes to using drugs, such as benzodiazepines, particularly early in development. Could more selective $\alpha2/\alpha3$ $GABA_AR$ agonists offer a better efficacy profile for very young patients, given the lower levels of $\alpha1$ subunit in many brain regions? Could selective enhancers of KCC2 enhance the therapeutic benefit of GABAergic agonists in very young patients? Would methods of tailoring such therapies to deliver them to the specific target areas, for the specific duration of the seizure, or other targeted symptoms prove to be safer? It should be noted, however, that most of the cited studies raising the concern that GABAergic drugs may have adverse effects in the naïve developing brain have been performed in naïve animals. There is no sufficient evidence to indicate that this may also be true for brains that have been exposed to GABAergic drugs with the intent to stop seizures, and it could prove to be challenging to design such a controlled study, given that benzodiazepines have been established as a first-line rescue treatment at all age groups. Yet, as we go forward and more clinical studies are being developed to test the efficacy and tolerability of benzodiazepines as rescue treatments for seizures, it would be worth evaluating not only the immediate efficacy and tolerability but also the longer-lasting consequences of exposure to GABAergic drugs, in both developmental and behavioural outcomes (Galanopoulou, 2008c). The experience in the NEMO (NEonatal seizures using Medication Off-patent) study with the observed ototoxicity from the use of bumetanide, an NKCC1 inhibitor, as adjunctive medication to barbiturates to stop neonatal seizures highlights the concern of safety (Pressler *et al*., 2013). This is particularly true for regions that are not necessarily within the epileptogenic zone and may become exposed to the drug. □

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# Treating acute seizures with benzodiazepines: does seizure duration matter?

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**ABSTRACT** – Several clinical trials have shown improved seizure control and outcome by early initiation of treatment with benzodiazepines, before arrival in the emergency department and before intravenous access can be established. Here, evidence is provided and reviewed for rapid treatment of acute seizures in order to avoid the development of benzodiazepine pharmacoresistance and the emergence of self-sustaining status epilepticus. Alterations in the physiology, pharmacology, and postsynaptic level of GABA-A receptors can develop within minutes to an hour and hinder the ability of synaptic inhibition to stop seizures while also impairing the efficacy of GABAergic agents, such as benzodiazepines, to boost impaired inhibition. In addition, heightened excitatory transmission further exacerbates the inhibitory/excitatory balance and makes seizure control even more resistant to treatment. The acute increase in the surface expression of NMDA receptors during prolonged seizures also may cause excitotoxic injury, cell death, and other pathological expressions and rearrangements of receptor subunits that all contribute to long-term sequelae such as cognitive impairment and chronic epilepsy. In conclusion, a short window of opportunity exists when seizures are maximally controlled by first-line benzodiazepine treatment. After that, multiple pathological mechanisms quickly become engaged that make seizures increasingly more difficult to control with high risk for long-term harm.

**Key words:** GABA-A receptor trafficking, NMDA receptor trafficking, seizure, status epilepticus, epilepsy, hippocampus

## Background: clinical perspective

Acute repetitive or prolonged seizures are some of the most common neurological emergencies presenting to the emergency department and can rapidly progress to status epilepticus (SE), with a mortality that approaches 23% (DeLorenzo *et al.*, 1996). Prolonged seizures themselves also may be harmful (Berg *et al.*, 1996; Dube *et al.*, 2010). Many factors contribute to the high morbidity and mortality and include aetiology (especially anoxia) and seizure duration, though it is often difficult to separate the effects of each (Maytal *et al.*, 1989; Lowenstein and Alldredge, 1993; Towne *et al.*, 1994; DeLorenzo *et al.*, 1996; Treiman *et al.*, 1998). However, several studies have shown that seizure duration

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is an independent adverse predictor of outcome (Lowenstein and Alldredge, 1993; Towne *et al*., 1994).
In addition, the longer the duration of a seizure, the more likely it is to continue (Scott *et al*., 1999; Shinnar *et al*., 2008), with an increasing likelihood of a poor outcome. In particular, 80% of seizures with a duration of less than 30 minutes respond to treatment while less than 40% seizures with a duration of greater than two hours respond well (Mayer *et al*., 2002). Also, seizures with a duration of greater than one hour are predictive of poor outcomes in all aetiological categories (Towne *et al*., 1994). After seizing for two hours, much damage may have already been done because no significant worsening of outcome is noted beyond that (Mayer *et al*., 2002).
Several long-term sequelae may result from prolonged seizure activity. These include neuronal death by 30-60 minutes of seizures (Meldrum and Horton, 1973), hippocampal injury (Ben-Ari, 1985; Cavalheiro *et al*., 1991; Fountain and Lothman, 1995; Cavalheiro *et al*., 1996), as well as a liability for chronic epileptic seizures. For example, status epilepticus increases the risk of spontaneous seizures in patients by 2.9 times (Walker, 1998), and seizure duration correlates with the development of spontaneous seizures in animal models (Dube *et al*., 2010). In addition, epilepsy occurs in 88% of cases of refractory SE but only 22% of cases of SE that respond to therapy (Mayer *et al*., 2002). Persistent disabling cognitive dysfunction also occurs in 11% of patients (Claassen *et al*., 2002), and animal studies reveal cognitive dysfunction associated with MRI hippocampal signal abnormalities in adults after prolonged febrile seizures as pups (Dube *et al*., 2009).
Seizures rapidly evolve (over minutes) through several stages as they become increasingly refractory to pharmacotherapy (Walton and Treiman, 1988; Treiman *et al*., 1998). They become self-sustaining by 20 minutes, paralleling the emergence of benzodiazepine resistance. While all seizures at early stages stop within two minutes of benzodiazepine treatment, the response rate rapidly decreases to less than 50% for more prolonged seizures. The results suggest an evolution of seizures that occurs over minutes.
Given the potential harm of prolonged seizure activity and the need for early recognition and treatment, an operational definition of status epilepticus (SE) as seizure activity greater than five minutes has been adopted by many for adult patients (Lowenstein and Alldredge, 1998; Lowenstein, 1999; Lowenstein *et al*., 1999). Activity that persists for greater than five minutes greatly exceeds (by more than two s.d.) the duration of a typical seizure and is unlikely to spontaneously arrest. Early treatment would shorten duration and help avoid adverse effects from prolonged seizure activity.
Some differences may exist for paediatric populations. First, although the majority of seizures that spontaneously abort will do so by five to ten minutes, up to 20% of both afebrile and particularly febrile seizures can spontaneously arrest after much longer durations, often exceeding 30 minutes (Hesdorffer *et al*., 2011). Lower morbidity also may be associated with these prolonged seizures compared to adults (Dunn, 1988; Maytal *et al*., 1989) and, unlike adults, rat pups that experience SE do not develop spontaneous recurrent seizures later (Zhang *et al*., 2004). In addition, SE is not associated with a rise in neuron-specific enolase (NSE) (a marker of neuronal injury) in pups while large elevations are noted in adults, and this correlates with histological evidence of damage (Sankar *et al*., 1997). Developmental differences in GABA-AR (and/or glutamatergic) subunit expression may contribute to these age-deterministic effects (Kapur and Macdonald, 1999; Zhang *et al*., 2004; Brooks-Kayal, 2005).
Despite possible age-related differences, the benefit of treating early (when treatment is most effective), in order to ensure seizure duration is short and avoid adverse outcomes, appears to outweigh potential risks such as respiratory depression, somnolence, and ataxia (Dreifuss *et al*., 1998; Kutlu *et al*., 2003). In fact, respiratory depression occurs more commonly in untreated patients who continue to seize (23%) compared to those who receive benzodiazepines in the field (10%) (Alldredge *et al*., 2001), and is not described as an adverse event in other studies (Knudsen, 1979; Dreifuss *et al*., 1998; McIntyre *et al*., 2005).
Benzodiazepines are the preferred initial therapy by neurologists and neuro-intensivists (Brophy *et al*., 2012; Riviello *et al*., 2013) for many reasons that include: rapid attainment of peak serum concentrations and onset of action/efficacy (within minutes), good CNS penetration, long duration of action (Leppik *et al*., 1983; Working Group on Status Epilepticus, 1993; Treiman *et al*., 1998), safety, and ease of use that includes the choice of multiple formulations and routes of access with high bioavailability, especially for water-soluble agents such as midazolam (Schwagmeier *et al*., 1998; Scott *et al*., 1999; Kutlu *et al*., 2003; Mahmoudian and Zadeh, 2004; McIntyre *et al*., 2005; Scott, 2005). However, clinical and animal studies show a rapid reduction in the potency of benzodiazepines with increasing seizure duration. While more than 60% of patients who present earlier with overt motor SE respond to lorazepam, less than 20% of those who present later with subtle SE achieve control of acute seizures (Treiman *et al*., 1998; Mayer *et al*., 2002). Buccal midazolam has a 100% response rate in children whose seizures are treated within 30 minutes, though only 50% respond beyond that time point (Kutlu *et al*., 2003). Also, the efficacy of rectal diazepam is significantly

attenuated after 15 minutes of seizures (Knudsen, 1979; Scott, 2005).

For several different animal models, the efficacy of benzodiazepines to stop seizures drops by 50% or more by 10-15 minutes (Walton and Treiman, 1988; Jones *et al.*, 2002), with most anticonvulsant drugs including GABAergic agents becoming completely ineffective by 35 minutes (Morrisett *et al.*, 1987). A 10-20 fold decrease in benzodiazepine potency is noted at this time (Kapur and Macdonald, 1997; Mazarati *et al.*, 1998a), and this is associated directly with alterations in the pharmacology of GABA-ARs that effectively render them unresponsive to benzodiazepines (Kapur and Macdonald, 1997).

Because the transit time to the emergency department (ED) often exceeds 15 minutes with a mean seizure duration exceeding 30 minutes upon arrival to the ED in one study (Alldredge *et al.*, 2001) and closer to 1.3 hours in another (Mayer *et al.*, 2002), several clinical trials have explored ways to avoid unnecessary delays and initiate treatment before arrival in the ED. These have included out-of-hospital delivery of benzodiazepines by paramedics or carers instructed on the use of rescue therapy in high-risk patients (Alldredge *et al.*, 1995; Lowenstein and Alldredge, 1998; Scott *et al.*, 1999; Alldredge *et al.*, 2001; Silbergleit *et al.*, 2012). Other trials attempt to reduce time-to-treat further by avoiding delays associated with obtaining intravenous access (McIntyre *et al.*, 2005; Silbergleit *et al.*, 2012).

Minimizing delays due to patient transfer or access improves seizure control and outcome. Out-of-hospital lorazepam in adults shortens seizure duration prior to treatment and terminates seizures in 59.1% before ED arrival compared to spontaneous seizure arrest in 21.1%, and decreases intensive care unit (ICU) admissions by more than 50% (an effect that was independent of aetiology) (Alldredge *et al.*, 2001). In children, pre-hospital treatment with intravenous or rectal diazepam shortens seizure duration to 32 minutes compared to 60 minutes for those initially treated in the ED and also decreases the likelihood of recurrent seizures or intubation (Alldredge *et al.*, 1995). In addition, compared to the intravenous route, the intramuscular route shortened time-to-treat by more than three minutes and stopped seizures in 73.4% *vs.* 63.4% of patients by the time of ED arrival, raising the possibility that shortening the delivery time of benzodiazepines by even a few minutes may improve outcome (Silbergleit *et al.*, 2012).

Here, basic mechanisms are considered for the rapid development of self-sustaining seizures associated with an erosion of GABAergic inhibition and the development of benzodiazepine pharmacoresistance. The argument is supported, that early intervention is the most effective treatment to prevent prolonged seizures and their harmful effects.

## Seizure-induced trafficking of GABA-ARs with loss of synaptic inhibition and available sites for benzodiazepine action

Within one hour of lithium-pilocarpine-induced seizures in rats, a reduction of miniature inhibitory postsynaptic current (mIPSC) amplitude by 27% and area-under the curve (AUC) by 16% indicates a loss of synaptic inhibition mediated by postsynaptic GABA-AR in dentate gyrus (DG) granule cells (Naylor *et al.*, 2005) (*figure 1A*). Receptor kinetic models and mean-variance fluctuation analysis estimate that the number of postsynaptic GABA-ARs is decreased by 50% (Naylor *et al.*, 2005; Naylor, 2010), consistent with the correlation between mIPSC amplitude and number of synaptic receptors (Nusser *et al.*, 1997). Kinetic changes also occur that primarily involve an increase of mIPSC decay time (Naylor *et al.*, 2005; Goodkin *et al.*, 2005; Feng *et al.*, 2008) and suggest alterations of GABA-AR

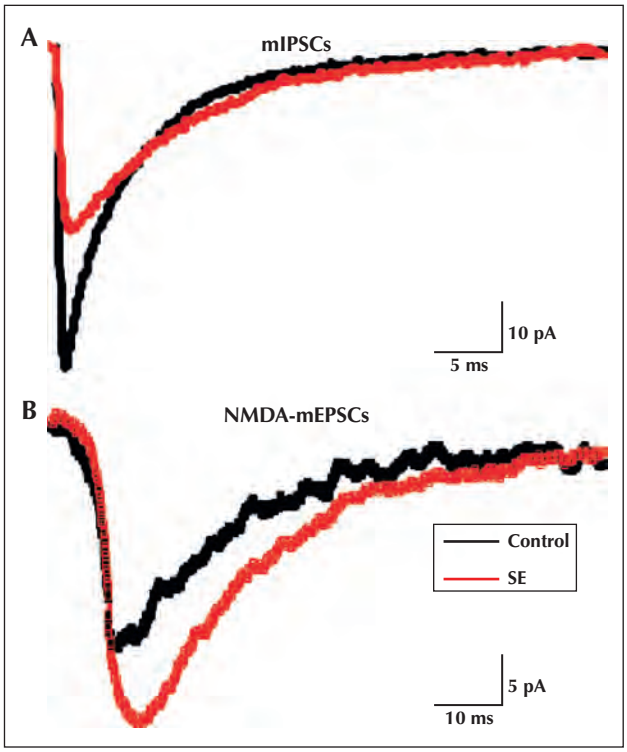


**Figure 1.** mIPSCs and NMDA-mEPSCs from dentate gyrus granule cells of SE and controls. (**A**) mIPSC mean traces from a typical granule cell from a control (black) and a SE animal (red), demonstrating smaller amplitude and prolonged decay in the latter. The mIPSCs were obtained by visualized whole-cell patch-clamp techniques with CsCl in the recording electrode and $V_{clamp}$ at -70 mV. (**B**) NMDA-mEPSC mean traces from a typical granule cell from a control (black) and a SE animal (red), demonstrating larger amplitude in the latter. The NMDA-mEPSCs were obtained by visualized whole-cell patch-clamp techniques with Cs gluconate in the recording electrode and $V_{clamp}$ at -60 mV.

functional properties, in addition to the decrease in postsynaptic receptor numbers.

Immunocytochemical labelling of the gamma 2 subunit, used to synaptically locate GABA-ARs (Nusser *et al.*, 1998) and associated with the synaptic clustering molecule gephyrin (Essrich *et al.*, 1998), confirms the decrease in the expression of synaptic receptors predicted by physiological measurements (Naylor *et al.*, 2005). The gamma 2 subunit also confers benzodiazepine sensitivity of synaptic GABA-ARs (Saxena and Macdonald, 1996); benzodiazepines bind at the pocket between alpha and gamma 2 subunits (Nusser *et al.*, 1998; Venkatachalan and Czajkowski, 2012), and gamma 2 is essential for benzodiazepine sensitivity (Pritchett *et al.*, 1989; Sigel *et al.*, 1990). Consequently, the loss of synaptic gamma2 subunit-containing GABA-ARs would be expected to decrease the number of available receptors for benzodiazepine binding and action.

The remaining synaptic GABA-ARs have a similar response compared with controls to maximal doses of diazepam, with a prolongation of mIPSC decay time and increase in AUC. But, the augmentation of synaptic inhibition by the benzodiazepine still remains insufficient to counter the initial loss by GABA-AR trafficking away from synapses during prolonged seizure activity (Naylor *et al.*, 2005). A similar study in juvenile rats shows diazepam responsiveness early and 30 minutes after acute seizures, though some blunting of the benzodiazepine response is noted at 30 minutes (Feng *et al.*, 2008). Whether or not direct alterations of GABA-AR function and pharmacology are contributory (Kapur and Macdonald, 1997; Feng *et al.*, 2008), and mIPSC kinetic changes after seizures do suggest GABA-AR functional changes, dramatic losses of synaptic gamma 2 subunit-containing GABA-ARs appear to be a major factor in the development of benzodiazepine insensitivity.

## NMDAR trafficking to synapses rapidly increases excitation

Unlike synaptic GABAergic inhibition, glutamatergic excitation increases in DG granule cells by one hour of lithium-pilocarpine-induced seizures. An increase of NMDA-mEPSC amplitude and AUC to 123 and 132%, respectively, of controls (*figure 1B*) is estimated (by mean-variance fluctuation analysis) to involve a 38% increase in the number of postsynaptic NMDARs (Naylor *et al.*, 2013). NR2B subunit-containing NMDAR primarily account for the increase, and immunocytochemical labelling of NMDAR subunits confirms trafficking of receptors to synapses.

An increased contribution of non-NMDARs to mEPSCs also occurs by one hour with an increase of amplitude to 120% of controls and estimated increase of 22% in the number of non-NMDARs at synapses (unpublished results). AMPAR potentiation is noted after hypoxic seizures as well (Rakhade *et al.*, 2008; Rakhade *et al.*, 2012), and seizure-induced switches of AMPAR subunit composition to Ca++ permeant, GluA2-lacking, variants also sustains seizure activity (Rajasekaran *et al.*, 2012). This augmented excitation in the background of degraded synaptic inhibition will further upset the balance between inhibition and excitation and greatly diminish the prospect for seizure control by benzodiazepines and other anticonvulsants.

In addition, NMDARs contribute to the downregulation of GABA-ARs, either as the result of circuit hyperactivity or direct NMDAR activation (Bannai *et al.*, 2009; Muir *et al.*, 2010), *via* calcineurin phosphatase action on gamma 2 subunits (Wang *et al.*, 2003; Muir *et al.*, 2010) and lateral diffusion of GABA-ARs away from synapses and potentially towards endocytotic sites (Wang *et al.*, 2003; Bannai *et al.*, 2009). These changes affect the synaptic, gamma 2 subunit-containing, and benzodiazepine sensitive, GABA-ARs. Pretreatment with NMDAR antagonists prevents the acute loss of synaptic inhibition (Kapur and Lothman, 1990) and the loss of benzodiazepine sensitivity, even after 60 minutes of seizures (Rice and DeLorenzo, 1999). Similarly, seizure-related AMPAR activation also down-regulates synaptic inhibition *via* calcineurin activation (Sanchez *et al.*, 2005).

## Activity-dependent and immediate functional losses of synaptic inhibition

Prolonged decay times of mIPSCs suggest functional alterations of postsynaptic GABA-ARs after one hour of seizures (*figure 1A*), but extracellular field recordings in the DG show that loss of evoked paired-pulse inhibition (PPI), another metric of synaptic inhibition, occurs after as little as one minute of perforant path electrical stimulation *in vivo*, and persists for greater than 20 minutes before recovery (Naylor and Wasterlain, 2005) (*figure 2A*). A similar loss of PPI for evoked postsynaptic GABA-AR responses recorded in DG granule cells occurs immediately after five minutes of stimulation *in vitro* (*figure 2B*). Because the loss of inhibition with electrical stimulation *in vivo* occurs well before the occurrence of isolated seizures and certainly before the 30 minutes of perforant path electrical stimulation necessary for self-sustaining seizures (Mazarati *et al.*, 1998b), diminished synaptic inhibition appears to precede the onset of seizures and the trafficking of GABA-AR associated with SE (Naylor *et al.*, 2005).

In addition, GABA-AR trafficking decreases of postsynaptic receptors would be expected to proportion-

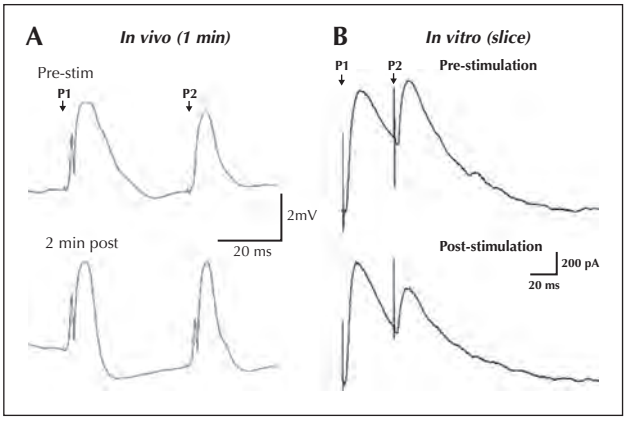


**Figure 2.** Loss of paired-pulse inhibition (PPI) measured in the DG after brief perforant path stimulation *in vivo* and *in vitro*. (**A)** Loss of PPI measured by field recordings *in vivo* with excitatory population-spikes on both P1 and P2 appearing within minutes after stimulation for one minute of 2-Hz continuous and 20 Hz for 10 sec/min. (**B)** Evoked IPSC responses recorded from the same DG granule cell before (top) and after (bottom) five minutes of repetitive perforant path stimulation (PPS).
Note greater inhibition with P2 before stimulation, but less after. Cs gluconate was in the recording electrode with $V_{clamp}$ at 0 mV.

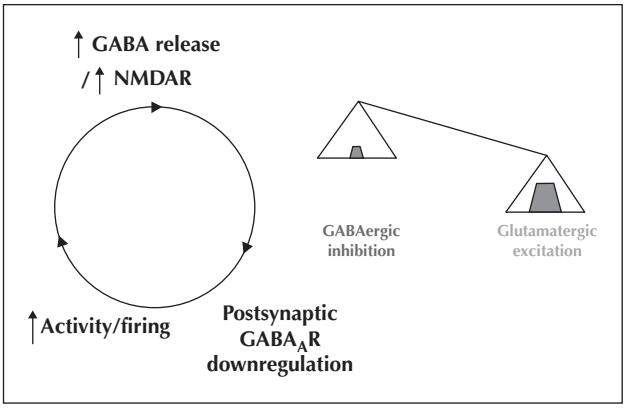


**Figure 3.** Schematic relating loss of inhibition to self-sustaining seizures and unbalanced glutamatergic excitation. Initial perturbation at any point in the cycle can lead to increase circuit activity with activation of NMDARs that trigger decreases of postsynaptic GABA-AR level and function. The erosion of inhibition further upsets the balance and supports persistent over-activity and seizures.

ately decrease the amplitudes of all evoked responses in proportion to the number of receptors lost and not change the ratio or interaction between pairs of evoked responses. The early loss of PPI implies a functional loss of synaptic inhibition before the absolute loss associated with postsynaptic GABA-AR decreases. This early loss of inhibition after brief convulsant-like stimulation may be sufficient to support spontaneous seizure activity (Kapur and Lothman, 1989), and spontaneous seizures maintain a loss of inhibition (Kapur *et al*., 1989), thereby perpetuating a "vicious cycle" of sustained loss of inhibition and ongoing seizures (*figure 3*).

## Seizures increase extracellular GABA

Along with the reduction of mIPSC currents, an increase of tonic GABA-AR currents occurs in DG granule cells after seizing for one hour (*figure 4A*). GABA-ARs are pentomeric structures primarily involving the co-assembly of three subunits: two alphas, two betas, and a gamma or delta subunit (McKernan and Whiting, 1996). The tonic currents are mediated by extrasynaptic GABA-ARs (Nusser *et al*., 1998) that, in DG, contain subunit subtype combinations that include a delta subunit as opposed to the gamma 2 subunit of synaptic receptors.
Unlike gamma 2 subunit-containing GABA-ARs, receptors with delta subunits have much less desensitization (*figure 5C*) and lack benzodiazepine sensitivity (Saxena and Macdonald, 1996; Knoflach *et al*., 1996; Haas and Macdonald, 1999; Brown *et al*., 2002). In addition, extrasynaptic receptors are much more sensitive to low levels of ambient GABA in the extracellular space. As a result of high sensitivity and low desensitization to GABA, extrasynaptic receptors can be reliable indicators of GABA levels. The mean and variance of extrasynaptic tonic currents correlates with GABA levels and can be used to generate a dose-response curve for extracellular GABA (*figure 4B*). Based on this curve, GABA-AR tonic currents predict that GABA levels can exceed 5 μM after one hour of seizing (Naylor *et al*., 2005). In fact, tonic currents after seizures are similar to those after added GABA (*figure 4A*).
Increase in the number of extrasynaptic GABA-ARs also could explain the increased tonic currents, and increased delta subunit expression has been described with SE by some (Terunuma *et al*., 2008), but not others (Goodkin *et al*., 2008). Because the addition of 100 μM GABA occludes the difference in tonic currents between SE and control DG granule cells (Naylor *et al*., 2005), the increase with SE is attributed to an increase in extracellular GABA more than to an increase in the number of extrasynaptic receptors. Regardless of whether some change in extrasynaptic delta subunit surface expression occurs during SE, our results support micromolar increases in extracellular GABA (Naylor *et al*., 2005), and steady increases have been observed more directly with assay measurements of GABA at various time points after the onset of seizures (Walton *et al*., 1990; Wasterlain *et al*., 1993). Also, even brief stimulation may increase tonic currents. DG granule cell tonic currents increase 18.9±4.8 pA ($p<0.05$) after five minutes of hyperstimulation *in vitro* (*figure 4C*) and follow a dose-response

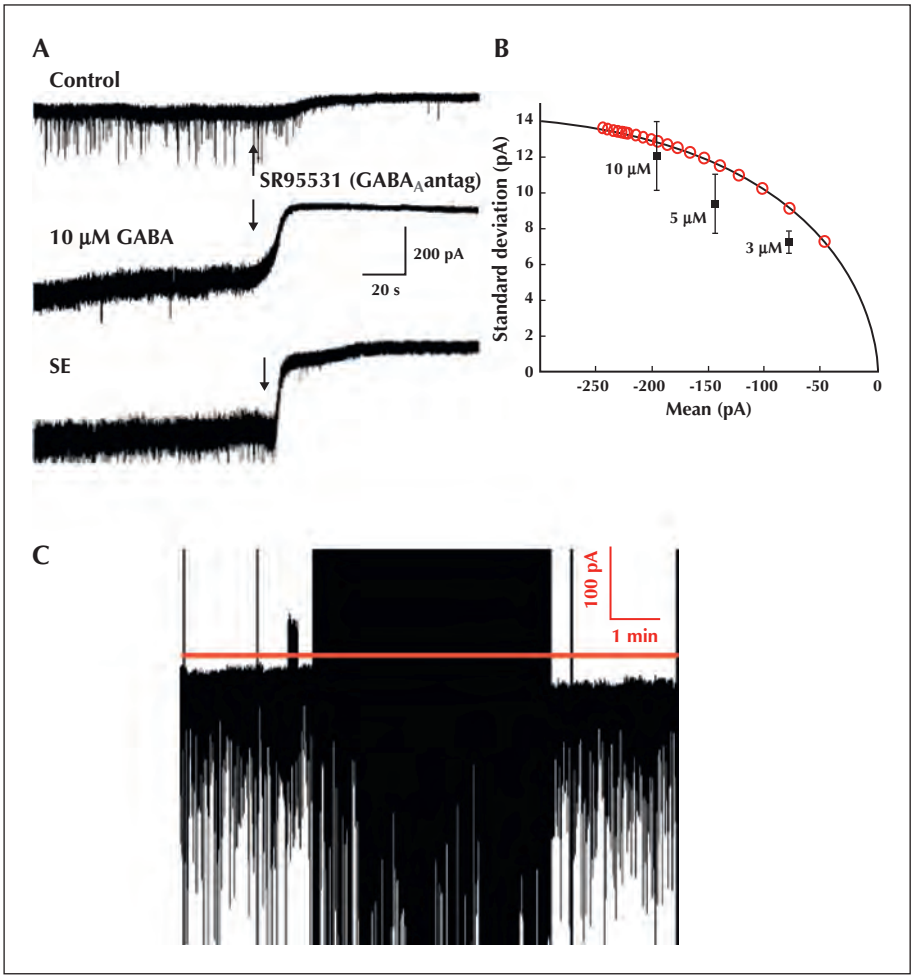


**Figure 4.** GABA-AR tonic currents recorded from DG granule cells. (**A)** Tonic current recordings from typical cells from control slices, slices bathed in elevated concentrations of extracellular GABA (10 µM), and slices after one hour of SE. Note the increase mean (and baseline standard deviation) of the tonic current with 10 µM GABA and SE compared to controls, as revealed by the greater baseline shift with addition of the GABA-AR antagonist SR95531. The increase in tonic currents after SE is consistent with increases in extracellular GABA. All recordings with GABA uptake inhibition (N0711; 10 µM). CsCl was in the recording electrode with $V_{clamp}$ at -70 mV. (**B)** Dose-response curve for the mean and standard deviation of GABA-AR tonic currents calibrated for known concentrations of added GABA then used to estimate extracellular GABA after SE or perforant path stimulation (see Naylor *et al.* [2005] for methods). Round red circles represent 1-µM increases in extracellular GABA (to a total of 20 µM). Boxes with error bars: ±SEM. (**C)** Small but significant increases in GABA-AR tonic currents occurred after five minutes of perforant path stimulation *in vitro* and are best visualised using the red baseline as a reference.

curve consistent with up to a micromolar elevation in the extracellular GABA (*figure 4B*), qualitatively similar, but less than is observed after one hour of SE (*figure 4A*). Tonic extrasynaptic GABA currents in the DG appear to parallel levels of circuit activity, which occurs in the cerebellum (Brickley *et al.*, 1996).

Sources of GABA may derive from synaptic release (Glykys and Mody, 2007), but also may occur from reversals of GABA transport by glia (Wu *et al.*, 2007). Certainly, an increase in synaptic release with circuit hyperactivity is expected during prolonged seizures, and blockade of GABA uptake after SE causes an increase, not a decrease, of GABA (as indicated by the increase in tonic currents) (Naylor *et al.*, 2005).

## GABA exposure (tonic or phasic) desensitizes and functionally alters synaptic GABA-ARs with early loss of paired-pulse inhibition

It is estimated that activity-dependent increases in extracellular GABA can exceed a few micromolar, especially after prolonged seizures. Moreover, adding micromolar amounts of GABA is sufficient to cause significant, rapid, and reversible desensitization of postsynaptic GABA-ARs (*figure 5A*) (Naylor, 2010), especially if uptake mechanisms are blocked and extracellular GABA can readily invade synapses and affect

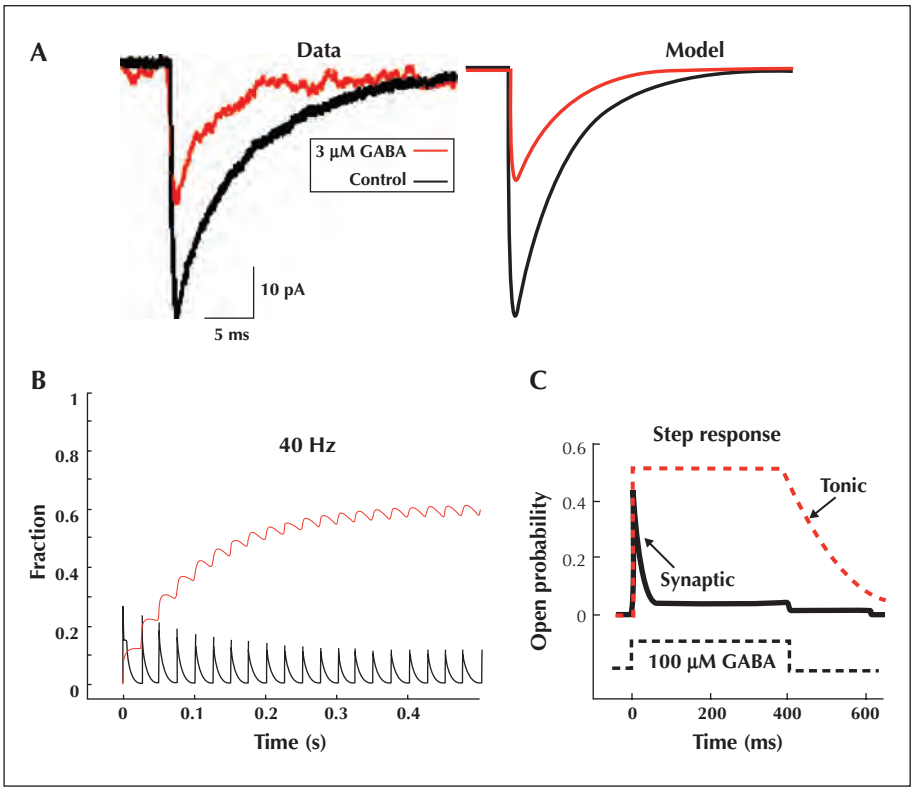


**Figure 5. (A)** Desensitization of synaptic GABA-ARs with addition of 3-µM GABA with a reduction of mIPSC amplitude of nearly 50%. GABA-AR receptor kinetic model prediction matches experimental results (see Naylor *et al.* [2005] for computational methods). **(B)** Model simulation of postsynaptic GABA-AR responses to high-frequency transmitter release. At 40-Hz stimulation for 200 ms, a greater than 50% loss of postsynaptic GABA-AR mIPSC is predicted (black) with nearly 50% of receptors entering desensitized states (red). (**C**) Predicted GABA-AR responses to step increases of GABA showing rapid and complete desensitization of receptors at synapses compared to extrasynaptic receptors that mediate tonic inhibition.

IPSCs. Exposure of desensitizing synaptic receptors to elevated levels of GABA may explain some reports of paradoxical worsening of seizures after treatment with tiagabine (Walton *et al.*, 1994; Shinnar *et al.*, 2001; Fitzek *et al.*, 2001), as well as instances of benzodiazepine ineffectiveness as treatment for these precipitated seizures (de Borchgrave *et al.*, 2003).

In addition, physiologically based synaptic models indicate that desensitization of postsynaptic GABA-ARs also occurs with brief high-frequency pulsatile exposure that simulates direct synaptic release under overactive conditions (using GABA-AR receptor kinetic parameters defined previously [Naylor *et al.*, 2005]). At 40 Hz, predicted desensitization of postsynaptic GABA-ARs will degrade IPSCs by more than 50% and this occurs after only 100-200 msec (*figure 5B*), raising the possibility that even very brief hyperactivity such as "fast ripples" could have an impact on GABA-AR properties.

Such rapid desensitization of gamma 2 containing receptors to high-frequency pulses of GABA has been observed with *in vitro* expression systems (Bianchi and Macdonald, 2002). In fact, exaggerated pulsatile release of GABA at synapses may desensitize postsynaptic GABA-ARs more potently than extracellular elevations of transmitter, especially when GABA uptake mechanisms are intact. GABA transporters, though numerous, have low turnover rates and regulate low (micromolar) levels of extracellular tonic (Jensen *et al.*, 2003; Hu and Quick, 2008) and spillover (Wei *et al.*, 2003) of GABA better than they can shape and control the high phasic concentrations (millimolar) of transmitter inside the synaptic cleft. Extrasynaptic delta subunit-containing receptors are more likely to be influenced by this type of transporter control, while synaptic gamma 2 subunit-containing GABA-ARs may remain vulnerable to circuit hyperactivity and desensitization. Desensitization from tonic GABA exposure or pulsatile release can contribute not only to an effective loss of available GABA-ARs (with decrease mIPSC amplitude; *figure 5A*), but also can alter postsynaptic receptor kinetic properties, including a loss of evoked PPI (*figure 6C*). Based on computational models of evoked IPSCs as a filtered sum of individual mIPSCs (with synaptic mIPSC representations and GABA-AR receptor kinetic parameters defined previously; Naylor *et al.*,

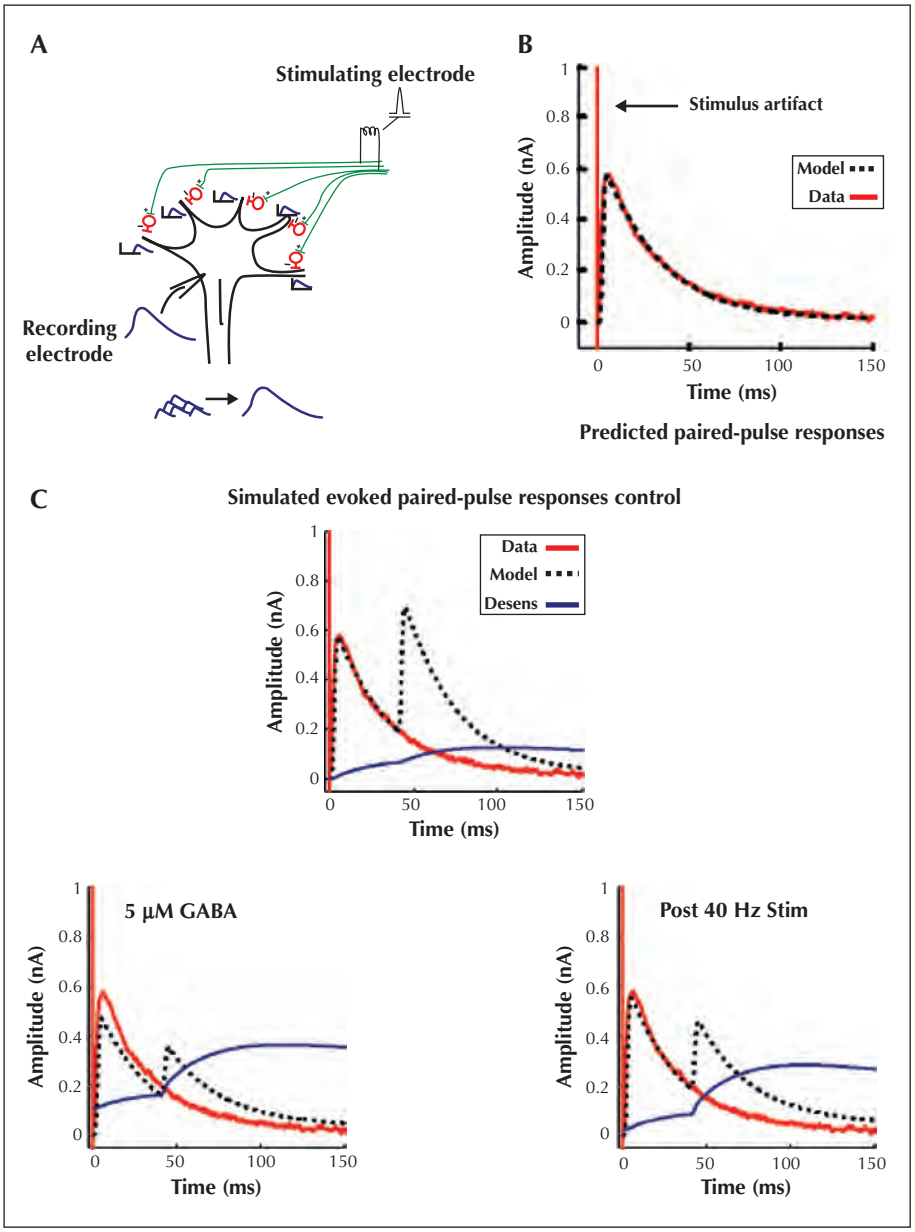


**Figure 6.** Perforant path evoked IPSCs from DG granule cells with simulated paired-pulse responses. (**A**) Schematic for the representation of evoked IPSCs as a filtered sum of individual synaptic events or mIPSCs. (**B**) A typical evoked IPSC recorded from a DG granule cell (red). Optimized model fit (black dot) of evoked IPSCs (previously described; Naylor *et al*., [2005]) defined parameters for paired-pulse simulations. (**C**) Simulated paired-pulse responses revealed intact PPI in controls with comparable losses of PPI with either 5-µM GABA exposure or after brief 40-Hz synaptic release. Loss of PPI is associated with hyperexcitability. Simulated paired-pulse responses correspond to postsynaptic GABA-AR contributions to loss of inhibition, not presynaptic effects on release probability. Cs gluconate was in the recording electrode with $V_{clamp}$ at 0 mV.

[2005]), simulations of evoked paired-pulse responses that apply parameters for GABA as either 5 µM of tonic or brief 40 Hz pulsatile GABA exposure show similar losses of PPI for either condition (*figure 6C*). A similar loss of PPI is observed experimentally after perforant path stimulation *in vitro* (*figure 2B*). Because desensitization occurs rapidly, it may cause very early losses of inhibition that occur during, or even precede, seizures (*figure 2*).

In addition, gamma 2 subunit-containing GABA-ARs at synapses have properties that include not only receptor desensitization but also sensitivity to benzodiazepines (Saxena and Macdonald, 1996; Haas and Macdonald, 1999). Therefore, desensitization

affects GABA-ARs that overlap with those that are benzodiazepine sensitive and could contribute to the development of benzodiazepine insensitivity even earlier than would occur from receptor trafficking. In addition, although GABA exposure and receptor desensitization is insufficient itself to induce synaptic GABA-AR trafficking (Goodkin *et al.*, 2008), it may be sufficient to trigger losses of inhibition with increases of DG circuit activity and stimulation of excitatory synaptic receptors. Activation of NMDARs (Bannai *et al.*, 2009) and AMPARs (Sanchez *et al.*, 2005) then can down-regulate synaptic GABA-ARs.

## Discussion

Both clinical and animal studies note a rapid loss of benzodiazepine potency as seizures persist (Kapur and Macdonald, 1997; Mazarati *et al.*, 1998a; Treiman *et al.*, 1998; Jones *et al.*, 2002), and this parallels the emergence of self-sustaining seizures with pronounced losses of synaptic inhibition. Several factors may be important. First, a decreased number of postsynaptic gamma 2 subunit-containing GABA-ARs occurs by one hour of seizures and is associated with reduced mIPSC amplitudes and selective trafficking of benzodiazepine-sensitive receptors (Naylor *et al.*, 2005; Terunuma *et al.*, 2008). Conversely, a rapid increase in the delivery of NMDARs containing NR2B subunits to the cell surface also occurs by one hour and contributes to increases in both synaptic and extrasynaptic excitation (Frasca *et al.*, 2011; Naylor *et al.*, 2013). This combination of effects causes an imbalance between inhibition and excitation that can sustain seizures and make them increasingly more difficult to treat (*figure 3*).

In addition, functional losses of synaptic inhibition in the DG occur within minutes of hyper-active perforant path stimulation and may involve rapid alterations of GABA-AR kinetic properties, such as desensitization (Naylor *et al.*, 2005; Naylor and Wasterlain, 2005; Naylor, 2010). Over-exposure to excess synaptic release and/or rising tonic levels of GABA may be sufficient to desensitize the gamma 2 subunit-containing and benzodiazepine-sensitive postsynaptic GABA-ARs.

The activation of NMDAR and Ca++ entry simultaneously may trigger pathways that shift GABA-AR surface expression away from synapses, further aggravating acute losses of synaptic inhibition (Wang *et al.*, 2003; Bannai *et al.*, 2009), while also contributing to long-term effects that include epilepsy (Rice and DeLorenzo, 1998), cognitive dysfunction (Dube *et al.*, 2009) and neuronal injury and cell death (Fujikawa, 1995; Deshpande *et al.*, 2008; Frasca *et al.*, 2011).

The multiple effects of seizures and circuit hyperactivity on GABA-ARs include some that change receptor functional properties (Kapur and Coulter, 1995; Kapur and Macdonald, 1997). Others, such as lateral diffusion of receptors away from synapses (Bannai *et al.*, 2009; Muir *et al.*, 2010) and trafficking of receptors to the cell interior (Naylor *et al.*, 2005; Goodkin *et al.*, 2005; Terunuma *et al.*, 2008), primarily change the number of receptors available at synapses and on the cell surface, although selective trafficking of particular subtypes of GABA-ARs could skew physiological and pharmacological properties based on changes in the proportion of receptor subtypes. In addition, the lateral diffusion of GABA-ARs away from synapses and closer to endocytotic zones may herald receptor trafficking.

These effects occur by one hour and some within minutes. For example, activity-dependent lateral diffusion of GABA-ARs decreases mIPSC amplitude by five to ten minutes, with recovery over a similar time course (Bannai *et al.*, 2009; Muir *et al.*, 2010). Functional alteration of synaptic inhibition associated with a loss of PPI, that initially may result from postsynaptic GABA-AR desensitization among other possibilities, also occurs within minutes and may recover within minutes or can persist longer, depending on the duration of seizure activity (Kapur and Lothman, 1989; Naylor *et al.*, 2002; Naylor *et al.*, 2005; Naylor and Wasterlain, 2005; Holtkamp *et al.*, 2005).

Many routes are available to initiate acute losses of synaptic inhibition. Heightened circuit activity, either by increases of excitation or by decreases of inhibition (Bannai *et al.*, 2009), leads to stimulation of NMDARs (Bannai *et al.*, 2009; Muir *et al.*, 2010) or AMPARs (Sanchez *et al.*, 2005; Rakhade *et al.*, 2008; Rakhade *et al.*, 2012). Calcineurin is a target of such activation with dephosphorylation of GABA-AR subunits and unmasking of AP2 binding sites for GABA-AR endocytosis (Bannai *et al.*, 2009). Alternatively, the activity of kinases including isoforms of PKC may be decreased (or increased) with similar results (Terunuma *et al.*, 2008). Changes in the phophorlylation state of GABA-AR subunits may not only alter the synaptic and cell surface numbers of receptors, but also can alter receptor physiological and pharmacological properties, including a loss of benzodiazepine sensitivity (Gao and Greenfield, 2005).

In addition, seizure-induced expression and potentiation of excitatory NMDARs (Frasca *et al.*, 2011; Naylor *et al.*, 2013) and AMPARs (Sanchez *et al.*, 2005; Rakhade *et al.*, 2008) will not only facilitate excitatory transmission and circuit hyperactivity, but also will further engage the same kinase and phosphatase transduction pathways that are responsible for GABA-AR down-regulation in the first place. The interaction

between NMDAR activation and GABA-AR regulation may explain why NMDA blockade prevents loss of benzodiazepine sensitivity as seizures progress (Kapur and Lothman, 1990; Rice and DeLorenzo, 1999).

Based on this scheme, a perturbation of inhibition or excitation could trigger circuit over-activity with movement of GABA-ARs away from synapses, loss of synaptic inhibition, and greater activity and stimulation of NMDARs (which is also increased by seizures). A "vicious cycle" of self-sustaining seizure activity could be established that preferentially drives the removal of postsynaptic gamma 2 subunit-containing GABA-ARs that are benzodiazepine sensitive.

Many anticonvulsants, including non-GABAergic drugs such as phenytoin, lose potency with prolonged seizures (Morrisett *et al*., 1987; Treiman *et al*., 1998; Jones *et al*., 2002), but benzodiazepines appear to be particularly affected (Walton and Treiman, 1988; Kapur and Macdonald, 1997). Effects on Cl- gradients that diminish GABA-AR-mediated hyperpolarisation occur during SE (Kapur and Coulter, 1995; Rivera *et al*., 2004; Lee *et al*., 2010) and certainly should diminish and possibly reverse the efficacy of benzodiazepines (Staley, 1992). However, such an effect should generalise to all drugs that act on GABA-ARs and to all GABA-ARs, including those at both synaptic and extrasynaptic sites.

However, even though the efficacy of GABAergic drugs as a class may be diminished with seizure progression, this is not uniform and differential effects have been noted between benzodiazepines, barbiturates, and propofol (Kapur and Macdonald, 1997; Treiman *et al*., 1998; Mayer *et al*., 2002; Rossetti *et al*., 2002; Shorvon, 2011). Prolonged seizures respond less well to benzodiazepines than barbiturates and propofol (Mayer *et al*., 2002; Rossetti *et al*., 2002), and GABA-ARs can become completely insensitive to benzodiazepines (Kapur and Coulter, 1995) while barbiturate sensitivity is preserved (Kapur and Macdonald, 1997). While treatment failure approaches 45% for midazolam in refractory SE, failure is indicated as 13 and 25% for barbiturates and propofol, respectively (Rossetti *et al*., 2002). A significant proportion of refractory SE responds to barbiturates after benzodiazepines have failed (Mayer *et al*., 2002).

A potential mechanism for differential loss of potency between benzodiazepines and barbiturates may relate to the preferred GABA-AR subunit binding sites for these agents and selective trafficking of GABA-ARs of particular subtypes during seizures. In particular, GABA-ARs with a gamma 2 subunit are synaptic (Nusser *et al*., 1998) and necessary for benzodiazepine sensitivity (Pritchett *et al*., 1989; Saxena and Macdonald, 1996). Benzodiazepines bind at the pocket between alpha and gamma subunits (Nusser *et al*., 1998; Venkatachalan and Czajkowski, 2012), while barbiturates and propofol bind the beta subunit that is ubiquitous for all GABA-ARs (Amin and Weiss, 1993; Serafini *et al*., 2000). Therefore, the movement of gamma 2 subunit-containing GABA-ARs away from synapses by lateral diffusion (Bannai *et al*., 2009; Muir *et al*., 2010) and/or receptor trafficking (Naylor *et al*., 2005; Terunuma *et al*., 2008) would preferentially affect the receptors with the greatest benzodiazepine sensitivity. Desensitization of the susceptible gamma 2-containing receptors (Haas and Macdonald, 1999; Bianchi and Macdonald, 2002) also may selectively restrict the availability of receptors with benzodiazepine sensitivity.

Pharmacoresistance may evolve from a combination of effects, both general and specific. The establishment, late into seizures, of self-sustaining hyperactive circuits, now characterised by alterations of both GABAergic and glutamatergic synapses, may be resistant to any intervention. However, there may be more specific effects of seizures, especially early, which focus on particular subtypes of GABA-ARs.

The subunit combinations of GABA-ARs evolve through brain development and may impact seizure characteristics, pharmacosensitivity, and long-term effects. In particular, alpha 1 and gamma 2 subunits, which combine to make up to 55% of GABA-ARs in mature synapses (Benke *et al*., 1994; McKernan and Whiting, 1996; Jacob *et al*., 2008), are at a low level at birth and increase two to three fold through adulthood (Brooks-Kayal *et al*., 1998; Brooks-Kayal, 2005). Receptors that contain this combination of alpha 1 and gamma 2 subunits are among the most benzodiazepine sensitive, with a seven fold increase in GABA efficacy (Pritchett *et al*., 1989), which explains the lack of benzodiazepine sensitivity in newborn rats (Kapur and Macdonald, 1999). In addition, the alpha 1 subunit is protective of seizures (Poulter *et al*., 1999; McIntyre *et al*., 2005; Raol *et al*., 2006), and mutations of alpha 1 and gamma 2 are associated with familial epilepsy (Bouthour *et al*., 2012). These developmental effects on GABA-AR subtypes may play a role in the longer duration of seizures in children compared to adults (Hesdorffer *et al*., 2011) as well as the high incidence of convulsive SE in children (Walker, 1998; Scott *et al*., 1999). Presumably, a lower expression level of GABA-ARs with combinations of alpha 1 and gamma 2 subunits could alter seizure responsiveness to particular pharmacological agents in an age-dependent manner as well.

Similarly, atypical GABA-AR subtypes with alpha 4 gamma 2 subunit combinations, that occur with epileptogenesis (Peng *et al*., 2004; Joshi and Kapur, 2013) and are benzodiazepine insensitive (Knoflach *et al*., 1996; Wafford *et al*., 1996; Brown *et al*., 2002), potentially could alter benzodiazepine responses in chronic epileptic patients, compared to new presentations.

When synaptic inhibition is lost and seizures become self-sustaining and benzodiazepine resistant, alternate therapies that might help restore the balance of inhibition and excitation include antagonists of NMDARs. NMDA blockade with ketamine or MK-801 is successful in several animal models of SE long after the development of benzodiazepine pharmacoresistance (Fariello *et al*., 1989; Walton and Treiman, 1991; Mazarati and Wasterlain, 1999; Borris *et al*., 2000).

Interestingly, NMDA antagonists may not be effective early or may even worsen seizures (Fariello *et al*., 1989; Bertram and Lothman, 1990), but may provide 100% control at 60 minutes (Borris *et al*., 2000). Perhaps inhibition from interneurons remains relatively intact early during seizures and NMDAR blockade not only decreases excitation of pyramidal and granule cells, but also decreases excitation of inhibitory interneurons, with disinhibition of pyramidal and granule cells. However, later after synaptic inhibition from interneurons has failed, the primary effect of NMDAR antagonism would be on excitatory cells. Combinations of NMDAR blockers and benzodiazepines may be much more effective than either agent alone (Walton and Treiman, 1991).

Clinically, success rates for treatment of SE by ketamine have been reported as high as 60-70% in epileptics (Rosati *et al*., 2012; Synowiec *et al*., 2013), but may be lower for refractory SE in patients with other aetiologies (Gaspard *et al*., 2013). An added benefit of treatment with NMDA blockers such as ketamine may not only be immediate seizure control, but also the prevention of long-term sequelae such as chronic epilepsy (Rice and DeLorenzo, 1998) and other adverse effects of excitotoxicity (Fujikawa, 1995; Deshpande *et al*., 2008; Frasca *et al*., 2011).

In conclusion, seizures rapidly become self-sustaining and pharmacoresistant secondary to multiple mechanisms that include: alterations of GABA-AR physiology and pharmacology, losses of synaptic GABA-ARs that mediate benzodiazepine action, and increases in the surface expression of excitatory NMDARs that make the task of restoring the balance between inhibition and excitation even more daunting. Very early treatment with a safe, fast, and effective drug, such as a benzodiazepine, before this intractable and deleterious sequence of events has opportunity to take hold, appears to be the best strategy. □

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# Are febrile seizures an indication for intermittent benzodiazepine treatment, and if so, in which cases?

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**ABSTRACT** – Febrile seizures occur in ~4% of children. After a first febrile seizure, the risk of recurrence is ~40%, but excellent studies document that febrile seizures do not cause brain damage or deficits in cognition or behaviour. The risk of subsequent epilepsy is 2-4%. Prolonged febrile seizures are of concern because a child may later develop mesial temporal sclerosis and intractable epilepsy in rare cases. Most prolonged febrile seizures represent the first febrile seizure and cannot be anticipated. A first prolonged febrile seizure does not increase the risk of recurrence, but if there is a recurrence, it is more likely to be prolonged. Prevention of recurrent febrile seizures is difficult. Antipyretics are ineffective. Daily AED treatment is not often justified. Intermittent oral diazepam at the time of illness is not very successful and has significant side effects. The most optimistic study found that the number of subjects required to treat in order to prevent one recurrence was 14. Intermittent clobazam has fewer side effects than diazepam and may be somewhat effective. Rescue benzodiazepines given outside health care facilities may be effective in selected patients to prevent prolonged recurrences, although this has not been proven with rectal diazepam which has been more extensively studied than buccal or nasal midazolam. Currently, we suggest that, for children with febrile seizures, candidates for consideration for rescue benzodiazepines are those with a prolonged febrile seizure or poor access to medical care. It is possible that the use of a rescue benzodiazepine may alleviate severe parental anxiety, but this remains to be established.

**Key words:** febrile seizure, recurrent seizure, prolonged, benzodiazepine

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Febrile seizures represent by far the most common convulsive event in humans. From birth to death, everyone faces, on average, an 8% risk of having some form of seizure and half of this risk corresponds to the chance of having a febrile seizure (Hauser and Kurland, 1975). Based on an overwhelming amount of evidence, primarily from population-based studies from various countries, the basic facts about febrile seizures are well-known (Nelson and Ellenberg, 1976; Verity *et al*., 1998; Annegers *et al*., 1987). About 3-6% of the population will

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have at least one febrile seizure at a peak age of 18 months, over a typical range of 4 months to 5 years. Children will occasionally have a febrile seizure at an older age. After a first febrile seizure, ~40% will have a recurrence. Few children will have three or more recurrences. Febrile seizures do not cause brain injury and are not associated with cognitive, personality, or behavioural changes. Specific issues concerning prolonged febrile seizures are discussed below. Children with "simple" febrile seizures have a 2-3% chance of later developing epilepsy. Those with complex febrile seizures (focal, prolonged, or repeated in the same illness) have a slightly increased risk (5-13%), but still, the vast majority do not develop epilepsy (Nelson and Ellenberg, 1976; Annegers *et al*., 1987).

On the other hand, several studies have shown that epilepsy in children is preceded by febrile seizures in ~15%, regardless of the epilepsy syndrome or the cause (Sofijanov, 1982; Camfield *et al*., 1994). This suggests that febrile seizures do not cause epilepsy, but rather, that the genes that determine the febrile seizure tendency are very important in determining a person's seizure threshold.

For this review, it is extremely important to realise that most, perhaps two thirds, of prolonged febrile seizures represent the first febrile seizure and that there is no way to anticipate them (Nelson and Ellenberg, 1976; Berg and Shinnar, 1996; Hesdorffer *et al*., 2013). Prolonged febrile seizures are of concern because of the observed sequence of a prolonged febrile seizure followed by intractable temporal lobe epilepsy caused by mesial temporal sclerosis. This sequence is relatively rare and has been estimated to occur in 1-2/150,000 children (Camfield *et al*., 1994). Following a very prolonged febrile seizure (most cases are longer that an hour), a recent study of 191 patients found that 10% showed mesial temporal T2 changes on MRI within 72 hours of their seizure, although we still do not know how many of these patients will develop MTS, temporal lobe epilepsy, or intractable temporal lobe epilepsy (Shinnar *et al*., 2012). The same MRI study showed that children with very prolonged febrile seizures also had an increased risk of partially malrotated hippocampus, a minor developmental anomaly. Interestingly, the malrotation was most often not on the same side as the T2 MRI changes.

The final concern for brain injury from febrile seizures is the hemiconvulsion-hemiplegia-epilepsy syndrome. Here, an extremely prolonged focal febrile seizure lasting hours is followed by permanent hemiplegia, contralateral brain hemiatrophy, and epilepsy (Tenney and Schapiro, 2012). This disastrous sequence has nearly vanished in developed countries, presumably as the result of aggressive treatment of status epilepticus.

There is no doubt that a major problem of febrile seizures is the upset they cause to parents. In studies from several countries, a very high proportion of parents from many different cultures reported that they thought their child was dying during their first febrile seizure (Balslev, 1991; Kolahi and Tahmooreszadeh, 2009). Understandably, parents are anxious about further illnesses. The effectiveness of education to allay these fears has not been well studied.

## Prevention of recurrent febrile seizures

Effective treatment to prevent recurrent febrile seizures has been elusive. Some of the issues are outlined in *table 1*. Remarkably, there is no benefit from aggressive antipyretic treatment at the time of illness (Offringa and Newton, 2013). Appropriate doses of acetaminophen, ibuprofen, and diclofenac have all been shown to be ineffective in double-blind trials.

Daily AED treatment seems unjustified, given the benign nature of febrile seizures. Daily phenobarbital, with its attendant behavioural and cognitive side effects, and valproic acid, with its risk of liver failure, may be effective, although several meta-analyses have contested this assertion (Masuko *et al*., 2003; Offringa and Newton, 2013). One meta-analysis suggested that the number of children required to treat with daily phenobarbital in order to prevent one febrile seizure was eight and with valproic acid was four (Rantala *et al*., 1997); carbamazepine and phenytoin appeared to be ineffective. More recent AEDs have simply not been studied. Benzodiazepines given intermittently at the time of illness may have some effect (*see below*).

## Intermittent treatment with benzodiazepines

Benzodiazepines can be given at the time of illness to try to prevent recurrence of febrile seizures or at the time of a febrile seizure to limit its duration. Whatever the drug and whatever the route of administration, there are several important caveats to this approach of treatment. First, the drug must be available at all times. This means that carers must have the drug with them at home, in the car, at day-care centres, and anywhere else where they might travel with the child. Second, the number of carers must be relatively small and all carers must be willing to administer the drug; this includes grandparents, babysitters, and day-care workers. At least in North America, for the majority of families, both parents are employed outside the house, so there are nearly always multiple carers for toddlers and preschool children. Third, all of those who might administer the drug must know how to do it accurately. All benzodiazepines have the potential to cause

**Table 1.** Issues for the treatment of febrile seizures

| **What should be the goals of treatment for febrile seizures?** |
|---|
| 1) Since there is some risk of brain injury from extremely prolonged febrile seizures, it would be desirable to prevent or shorten them. |
| 2) Since families are extremely upset by febrile seizures; treatments that reduce this anxiety might be beneficial. |
| **What are untenable goals of treatment?** |
| 1) Prevention of subsequent epilepsy |
| 2) Improvement of cognitive outcome |
| **What are the caveats for treatment with intermittent benzodiazepines?** |
| 1) Instant availability of the drug |
| 2) Number of carers |
| 3) Willingness of all carers to administer the drug |
| 4) Teaching carers |

significant sedation with too large a dose. Fourth, teaching carers about administering the drug should be carried out by health professionals. In our experience with 30 families who were instructed to give liquid rectal diazepam at home for prolonged seizures, we were very concerned to learn that, unbeknownst to us, 12 of the parents taught others how to give the diazepam (Camfield *et al*., 1989). We strongly discourage this practice for fear of overdose.

## Approaches to intermittent benzodiazepine treatment

There are two approaches to intermittent benzodiazepine treatment; treatment during illness and treatment once a seizure starts.

Treatment during illness has not been a very effective approach. The best known study involved oral diazepam at 0.33 mg/kg, given every 8 hours during illness (Rosman *et al*., 1993). This study randomised 406 patients with a first febrile seizure to receive oral diazepam or placebo at the time of illness and showed that the chance of a recurrence was marginally statistically reduced. The risk of recurrence was reduced from 31 to 23%. Unfortunately, about 30-40% of children receiving diazepam had significant side effects, including drowsiness and ataxia. These are very concerning symptoms considering their overlap with symptoms of meningitis which is always a concern for a child with a febrile seizure. No data was offered regarding the number of consultations with a physician or emergency room visits for these symptoms. We calculated that this regime would require treatment of 14 patients to prevent a single recurrent febrile seizure (Camfield *et al*., 1995). In the study, some patients took the diazepam exactly as per protocol and yet had a recurrence; oral diazepam is not 100% effective. No benefit was identified based on other randomised trials using a smaller dose of oral diazepam, therefore, reducing the dose to reduce side effects is not likely to be an effective strategy (Autret *et al*., 1990; Uhari *et al*., 1995).

A small, but intriguing, randomised study used oral clobazam ($n$=20) *versus* placebo ($n$=19) as an intermittent treatment and found clobazam to be very effective (Rose *et al*., 2005). A second randomised, small study compared intermittent clobazam ($n$=37) with intermittent diazepam ($n$=35) and found both drugs to be equally effective in preventing recurrences, but noted that 20 (54%) cases in the diazepam group and 5 (14.2%) in the clobazam group developed drowsiness and sedation ($p$=0.0001) (Khosroshahi *et al*., 2011). More investigations with intermittent oral clobazam may be warranted.

Given the benign nature of febrile seizures, it is unfortunate that no randomised study has examined the effect on parental anxiety of intermittent, preventative benzodiazepine treatment for their child. Are parents rendered more or less anxious by the need to frequently check the child's temperature and give medication with side effects? Would they be better off with a "grin and bear it" approach. In Nova Scotia, intermittent treatment is rarely offered, but we know that children with febrile seizures do not consume more health care resources than controls, and parents are not constantly rushing to the doctor with their child (Gordon *et al*., 2000).

The other approach is to offer benzodiazepines as "rescue" medication. The basis of this concept is that parents or carers give a dose of benzodiazepine only during an actual recurrent febrile seizure with a view to preventing a prolonged febrile seizure (Knudsen, 1996). This approach is limited to routes of administration with rapid absorption; rectal diazepam, rectal or sublingual lorazepam, or nasal/buccal midazolam. Rectal diazepam and buccal midazolam have been fairly extensively studied in emergency room settings and both are effective at stopping seizures (Rainbow *et al*., 2002). In a relatively recent randomised study of patients with prolonged seizures due to any cause in an emergency department, it appeared that buccal midazolam was superior to rectal diazepam; the first dose of buccal midazolam was successful in 56%, compared to 27% with rectal diazepam (McIntyre *et al*., 2005). There are advantages to buccal midazolam but rectal diazepam has been more systematically studied and it is likely that both are effective, however, both have some failings. Despite the intuitive nature of this treatment with benzodiazepines during a recurrent febrile seizure, there are no data from randomised trials to show a reduction in prolonged febrile seizures. One large case series suggested that rescue rectal diazepam did not completely alleviate parental anxiety (Rossi *et al*., 1989). All of the caveats for intermittent treatment to prevent febrile seizures apply to the approach of rescue medication during an actual seizure, with an extra need to be certain about correct dosing. Sublingual and rectal preparations can produce very high drug serum levels with the possibility of apnoea.

## Who might be a candidate for intermittent benzodiazepine treatment?

### Intermittent treatment to prevent a recurrence

In our opinion, oral diazepam is not sufficiently effective to be used to try to prevent recurrent febrile seizures. Effective doses mean significant side effects, and the prospect of treating 13 children unnecessarily to prevent a single febrile seizure in the fourteenth child is unpalatable. Oral clobazam needs further study. If oral preventative treatment is to be considered, there are some factors that make a recurrence more likely. These include a family history of febrile seizures, age at first febrile seizure <18 months, temperature at the time of the first febrile seizure <101°F, and a short illness before the seizure (<1 hour). In one prospective study of 428 children with a first febrile seizure, children with none of these four risk factors had a 14% risk of recurrence. With one factor, the risk was 23%, with two factors 32%, with three factors 62%, and with all four 76% (Berg *et al*., 1997). Some, but not all, studies have found that attendance at day-care centres increases the risk of recurrence as the result of increased infections. It is worth noting that these risk factors are quite different to the risk factors for epilepsy following a febrile seizure.

In addition, it is important to realise that a prolonged first febrile seizure does not increase the risk of a recurrent febrile seizure. However, if the first febrile seizure is prolonged (>10 minutes), the chance of a prolonged recurrence (>10 minutes) has been estimated at 20%, compared to a 6.8% risk of a prolonged recurrence if the first seizure was brief (Berg and Shinnar, 1996). This study was based on a prospective assessment of 118 patients with a first and then recurrent febrile seizure. If preventative intermittent therapy is to be considered, then it may make most sense to limit its use to those with three or four risk factors for recurrence. A randomised study of 139 children who received intermittent oral diazepam (0.33 mg/kg) or no benzodiazepines found that the greatest reduction in febrile seizure recurrence was in those at highest risk of recurrence (Pavlidou *et al*., 2006).

### Home use of a "rescue" benzodiazepine at the time of a recurrent febrile seizure

Since the most defensible goal of rescue medication is to prevent a prolonged recurrent febrile seizure, it would be very useful to identify who might be at risk. Unfortunately, as noted above, the only predictive factor appears to be a prolonged first febrile seizure. We believe that it is reasonable to offer home buccal midazolam or rectal diazepam to these families, although we emphasize that no study has shown the true value of this approach.

Other families may benefit from this approach, either based on a very itinerate lifestyle or limited access to medical care. For example, we often encounter families who travel with their children during holidays. They seem to gain in self confidence if they have a rescue medication available. Again, this has not been proven. Other families, especially in Canada, live in settings that are many hours travel from medical care. A very prolonged recurrent febrile seizure might be a disaster and therefore home treatment would seem justifiable. For families that are very anxious about febrile seizures, a rescue benzodiazepine may be helpful with all of the caveats mentioned above. The prescribing physician needs to monitor the apparent value of this treatment to be sure that it does not increase anxiety.

Should every family with a child with a first febrile seizure be offered home rescue benzodiazepines? In our opinion (and experience) the vast majority cope quite well and the facts about febrile seizures are so reassuring that more intervention does not seem justified. □

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Prolonged Epileptic Seizures:
identification and treatment

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# Syndromes at risk of status epilepticus in children: genetic and pathophysiological issues

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**ABSTRACT** – Status epilepticus (SE) is a medical emergency with increased risk of morbidity and mortality in all age groups. Recent research has identified a variety of new genes implicated in disorders with severe epilepsies as a prominent feature. Autoimmune mechanisms have also been recently recognised as a cause of epilepsies with SE as a characteristic symptom. Knowledge about the aetiology potentially underlying SE may help to guide diagnostics and eventually influence treatment decisions. This review recapitulates, in brief, the risk of SE in specific clinical settings, provides an overview of paediatric epilepsy syndromes more commonly, or by definition, associated with SE, and summarizes some recent research data on genetic defects and disease mechanisms implicated in the pathogenesis of epilepsies frequently accompanied by SE.

**Key words:** status epilepticus, prolonged seizures, childhood, etiology, classification, genetics

Status epilepticus (SE) is a medical emergency with increased risk of morbidity and mortality in all age groups. The glossary of descriptive terminology for ictal semiology by the International League Against Epilepsy (ILAE) defines SE as either a seizure which shows no clinical signs of terminating after a period of time corresponding to the duration of the great majority of seizures of that type in most patients, or recurrent seizures without resumption of baseline central nervous system function interictally (www.ilae.org/Visitors/Centre/ctf/glossary.cfm). Traditionally, SE has been defined as a seizure or series of seizures lasting longer than 30 minutes (Berg *et al*., 2004), while the term "prolonged seizures" has been used by several authors with slightly different definitions for seizures lasting more than five, but less than 30 minutes (Shinnar, 2007). It has been proposed using more recent guidelines that seizures lasting for 5 minutes or more are classified as SE, as most seizures last less than 5 minutes; seizures that last longer often do not cease spontaneously and irreversible neuronal damage and pharmacoresistance may occur within 30 minutes of seizure activity (Brophy *et al*., 2012). However, this view is still a matter of debate (Shinnar *et al*., 2001; Beran, 2008). In this review, we will adopt the traditional SE definition, whereas seizures lasting for more than 5, but less than 30, minutes will be connoted as prolonged seizures.

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Generalised convulsive SE is characterised by rhythmic jerking of all extremities and profound mental impairment (Brophy *et al*., 2012). Epilepsia partialis continua can be delineated from this form of status, since motor epileptic seizures are restricted to certain parts of the body (*e.g*. face, hands) and recur every few seconds or minutes for extended periods (more than one hour) (Cockerell *et al*., 1996; Bien and Elger, 2008). The term non-convulsive SE describes continuous seizure activity observed on electroencephalography (EEG), but without clinical findings associated with generalised convulsive SE (Brophy *et al*., 2012). In this form of status, seizure symptomatology is usually subtle and the semiological spectrum highly variable (Jirsch and Hirsch, 2007).

Apart from duration and semiology, SE can also be classified by its underlying aetiology. Some epileptic encephalopathies are, by definition, associated with SE, while prolonged seizures or SE are characteristic features of several epilepsy syndromes manifesting during infancy and childhood. New genetic techniques have identified a variety of new genes implicated in disorders with severe epilepsies as a prominent feature (Lemke *et al*., 2013). Autoimmune mechanisms have been recently recognised as potential causes of epilepsies. In some of these disorders, SE or prolonged seizures represent characteristic clinical symptoms (Davis and Dalmau, 2013).

Data on the recurrence risk of SE is important, since such information may influence therapeutic considerations. Knowledge about the aetiology potentially underlying SE may help to guide diagnostics and eventually influence treatment decisions. This review recapitulates, in brief, the risk of SE in specific clinical settings, provides an overview about paediatric epilepsy syndromes associated with SE, and summarizes some recent research data on genetic defects and disease mechanisms implicated in epilepsies frequently accompanied by SE.

## Risk of SE and/or prolonged seizures in specific clinical settings

### Risk of SE and prolonged seizures in children with febrile convulsions

More than 25% of children with febrile convulsions will experience at least one prolonged seizure lasting longer than 10-15 minutes. Febrile seizures with focal onset show a tendency to be prolonged (Berg *et al*., 1990). Recent results of the FEBSTAT study team revealed further factors that increase the risk of prolonged febrile convulsion and febrile SE: a preceding febrile convulsion occurring at an unusually young age (<12 months), low temperature, longer duration of fever, female sex, a positive first-degree family history of febrile convulsions, and structural temporal lobe abnormalities (Hessdorfer *et al*., 2013).

### Risk of SE and prolonged seizures in children with a first unprovoked seizure

In a prospective study by Shinnar and colleagues encompassing 407 patients, the first seizure lasted 5 minutes or more in 50%, 10 minutes or longer in 29%, more than 20 minutes in 16%, and 30 minutes or longer in 12% of cases. Seizures of partial onset had an increased tendency to be prolonged, compared to generalised seizures (20% *vs*. 6% with seizures >30 minutes, respectively) (Shinnar *et al*., 1990; Shinnar *et al*., 1996; Shinnar *et al*., 2000; Shinnar *et al*., 2001; Shinnar, 2007). Aetiology and age were not factors influencing seizure duration. Altogether, the authors concluded that two distinct subgroups of patients exist. One group, comprising about 75% of children, has seizures of short duration, while the other, consisting of approximately 25% of patients, shows a propensity for prolonged seizures. Mean seizure duration in the latter group was 30 minutes. Notably, the recurrence risk of seizures was not influenced by the duration of the first unprovoked seizure, although subjects with prolonged first seizures were more likely to suffer further prolonged seizures (Shinnar, 2007).

### Risk of SE in children with new-onset epilepsy

A population-based study from Finland in which 150 children with new-onset epilepsy for more than 30 years were followed, found that 27% of these patients suffered at least one SE. Of these children, 90% presented with a status within the first two years, and 73% within the first year after the diagnosis of epilepsy was established (Sillanpää and Shinnar, 2002). Risk factors for SE included young age and remote symptomatic aetiology. In children with a preceding SE, the risk of further prolonged seizures was increased by about 50%. Of note, the occurrence of a SE did not negatively affect the long-term prognosis of affected individuals (Sillanpää and Shinnar, 2002).

## Epileptic encephalopathies with propensity for prolonged seizures or SE

### Early myoclonic encephalopathy (EME)

EME is a rare epileptic entity with neonatal onset. Erratic and massive generalised or focal myoclonus in developing parts of the body is the clinical hallmark.

Persisting myoclonus during sleep is also characteristic. The jerks are extremely frequent and may even be continuous. Typically, the EEG shows a suppression-burst pattern. The aetiology is heterogeneous. Many patients suffer from autosomal recessive metabolic defects, such as non-ketotic hyperglycinaemia. Some authors count the Hanefeld variant of Rett syndrome with early-onset seizures amongst this entity. Partial seizures and spasms are observed during the later part of the course. Prognosis is dismal (Pavone *et al*., 2012).

### Early infantile epileptic encephalopathy (EIEE or Ohtahara syndrome)

EIEE is mainly caused by cortical malformations and, less frequently, by metabolic disorders. In some cases, defects in the *ARX*- or *STXBP1* genes may be detected (*table 1*). Single or serial tonic seizures, focal motor seizures, and generalised tonic-clonic seizures prevail. Single spasms last up to 10 seconds, and the intervals between spasms range from 9 to 15 seconds. As in EME, background EEG is characterised by a suppression-burst pattern. Surviving infants are usually quadriplegic and present with severe intellectual disability (Ohtahara and Yamatogi, 2003; Beal *et al*., 2012).

### West syndrome

West syndrome is characterised by the clinical triad of infantile spasms, developmental regression, and hypsarrhythmia. Spasms, lasting 2-5 seconds may occur in clusters of up to 100 every 5-30 seconds (Plouin *et al*., 1993). Tuberous sclerosis, congenital or acquired cerebral lesions, congenital infections, and rarely metabolic disorders are causative (Paciorkowski *et al*., 2011). Recently, genetic defects in the genes *ARX, CDKL5, SCN8A, STXBP1, DNM1*, and *GABRB3* have been reported in some of the patients (*table 1*).

### Malignant migrating partial seizures in infancy

The disease manifests from the first week of life to seven months of age. After an initial period lasting from a few days to months with relatively rare partial seizures that frequently have a motor or tonic component, seizure frequency dramatically increases and ictal semiology becomes highly polymorphous (Hahn *et al*., 2007). Typically, duration of seizures is between one and four minutes, but in some patients they may last significantly longer and evolve into tonic status epilepticus (Coppola, 2009). With time, head growth becomes arrested. Over the years, the epilepsy wanes. In some cases, retigabine has been surprisingly effective for some time. Defects in *KCNT1* are detectable in about 50% of cases (Barcia *et al*., 2012) (*table 1*).

### Dravet syndrome (or severe myoclonic epilepsy of infancy)

Dravet syndrome is characterised by onset of seizures in a febrile context within the first year of life in hitherto normally developed children. Prolonged unilateral seizures, often alternating in side, are generally the first type of seizures encountered. Frequent febrile status, myoclonic seizures, atypical absences, and later also focal seizures are characteristic. Valproate, topiramate, benzodiazepines, bromides, stiripentol, and a ketogenic diet are effective. However, save for rare exceptions, children do not become seizure-free for longer periods. Defects in *SCN1A* (up to 85% of cases), *SCN1B*, *SCN2A*, *PCDH19* and recently *CHD2* have been detected (Suls *et al*., 2013).

### Lennox-Gastaut syndrome (LGS)

LGS frequently follows West syndrome or other infantile epileptic encephalopathies. In many cases, cortical malformations are detectable. Atypical absences, sometimes lasting hours, non-convulsive status epilepticus, and tonic status epilepticus are common features. Defects in the genes *SCN8A*, *STXBP1*, *DNM1*, and *GABRB3* were recently recognised in some "idiopathic" cases with and without preceding West syndrome (Epi4K Consortium, 2013).

### Continuous spike and waves during slow-wave sleep and related encephalopathies

Atypical benign partial epilepsy (ABPE) is defined by seizures evocative of benign epilepsy with centro-temporal spikes (BECTS) in conjunction with atonic seizures and atypical absences. The EEG shows focal sharp waves as in BECTS, but with exceptional pronounced activation during sleep. Generalised seizures tend to be prolonged and may last for hours or days. A status of atypical absences/and or subtle myoclonic atonic seizures has been reported in up to 40% of children. As in BECTS, prognosis with respect to epilepsy is usually favourable, but some patients may be left with persistent mental deficits (Hahn, 2000).

Patients with epileptic encephalopathy and continuous spike and waves during slow sleep (CSWS) typically have relatively rare, mainly nocturnal, seizures in combination with a continuous bioelectrical status recorded on EEG due to drowsiness throughout all non-REM sleep stages. Major neurocognitive deficits

**Table 1.** Targets for genetic workup in children with prolonged seizures or status epilepticus.

| | |
|---|---|
| *ADSL* | Adenylosuccinate lyase deficiency is a rare cause of a severe epileptic encephalopathy starting within the first few days of life. Therapy resistance and SE are common (Jurecka *et al*., 2008). |
| *ALDH7A1* | Mutations result in pyridoxine-dependent seizures. Affected children usually present with SE early after birth, but sometimes distinctly later (Oliveira *et al*., 2013). |
| *ARX* | Defects are associated with a variety of cortical malformations (*e.g*. lissencephaly), additional organ manifestations, and intractable neonatal seizures in boys (Mirzaa *et al*., 2013). |
| *FOLR1* | Folate receptor defects are associated with neurological regression, movement disorder, hypomyelination on MRI and sometimes severe epilepsy. The disorder is rare, but potentially treatable (Steinfeld *et al*., 2009). |
| *FOXG1* | Mutations, deletions, or duplications may produce a congenital form of Rett syndrome in females and also in males, sometimes associated with severe epilepsy or atypical West syndrome (Guerrini and Parrini, 2012). |
| *GABRG2* | Deleterious defects may result in Dravet syndrome, while missense mutations cause milder phenotypes within the spectrum of idiopathic generalised epilepsies (Huang *et al*., 2012). |
| *GAMT* | Cerebral creatine deficiency syndrome due to GAMT deficiency may result in epileptic encephalopathies including Lennox Gastaut syndrome. The disorder is partially treatable (Mikati *et al*., 2013). |
| *GRIN2A* | Mutations are related to a spectrum of epilepsies ranging from Rolandic epilepsy to Landau-Kleffner syndrome or CSWS (Lesca *et al*., 2013; Lemke *et al*., 2013; Dimassi *et al*., 2014). |
| *KCNQ2* | Dysfunction of this ion channel causes benign familial neonatal seizures. Recent research revealed that the phenotype is much broader, and that defects in this gene accounts for about 10-15% of neonatal epileptic encephalopathies (Weckhuysen *et al*., 2013). |
| *KCNT1* | Defects result in malignant migrating partial seizures in infancy or severe autosomal dominant frontal lobe epilepsy (Barcia *et al*., 2012). |
| *KCTD7* | Mutations cause an autosomal recessive progressive myoclonus epilepsy of early childhood onset with refractory epilepsy (Van Bogaert *et al*., 2007). |
| *PLCB1* | Homozygous defects are a rare cause of malignant migrating partial seizures in infancy (Poduri *et al*., 2012). |
| *PNKP* | The gene product is implicated in a DNA repair mechanism. Recessive mutations result in microcephaly, early-onset intractable seizures, and developmental delay (Shen *et al*., 2010). |
| *TREX1*<br>*RNASEH2A*<br>*RNASEH2B*<br>*RNASEH2C*<br>*SAMHD1* | Defects in these genes account for the majority of cases with Aicardi-Goutières syndrome (Aicardi *et al*., 1993-2013). |
| *SCN1A+B*<br>*SCN2A*<br>*SCN8A+9A*<br>*CHD2*<br>*SYNGAP1* | Mutations in one these genes may lead to infantile epileptic encephalopathies which range from Dravet syndrome to Ohtahara syndrome (Carvill *et al*., 2013). |
| *PLCB1*<br>*SLC25A22* | Defects may result in infantile epileptic encephalopathy with suppression-burst pattern on EEG (Pavone *et al*., 2012). |
| *SPTAN1* | Mutations may cause West syndrome with cerebral hypomyelination (Saitsu *et al*., 2010). |

are frequent. In some patients with acquired epileptic aphasia (Landau-Kleffner syndrome), seizures reminiscent of BECTS are observed, while the EEG pattern during sleep frequently parallels the continuous spike-wave activity recorded in CSWS (Dalla Bernadina *et al*., 2002). Recently, mutations in the gene *GRIN2A*, encoding the α2 subunit of the *N*-methyl-D-aspartate (NMDA)-selective glutamate receptor, have been reported in patients affected by BECTS, ABPE, LKS, and CSWS (Lemke *et al*., 2013; Lesca *et al*., 2013; Dimassi *et al*., 2014).

### Autoimmune-mediated encephalopathies

Autoimmune mechanisms are increasingly recognised to play a causative role in the development of epilepsy in adults and children. Antibodies can be directed against intracellular structures or neuronal surface antigens. Autoimmune encephalopathies may manifest without underlying malignancies or present as paraneoplastic syndromes. In encephalopathies with paraneoplastic aetiology, anti-Hu antibodies are frequently associated with seizures, epilepsia partialis continua, and SE (Davis and Dalmau, 2013). Antibodies directed to the GABA(B) receptor are observed in cases with limbic encephalitis with early and prominent seizures (Lancaster *et al*., 2010). About 70% of patients with antibodies directed against the NR1 subunit of the N-methyl-D-aspartate receptor develop seizures and/or SE. Approximately 40% of cases manifest before the age of 18 years. Seizures or SE are the presenting sign in about 30% of these children. An underlying ovarian teratoma is detected in 50% of female patients older than age 12 years. Steroids, immunoglobulin, plasma exchange, and tumour removal, if indicated, are recommended as first-line therapies. Recovery is slow and may take many months, but most patients remain free of seizures (Davis and Dalmau, 2013).

Steroid-responsive encephalopathy associated with autoimmune thyroiditis (SREAT), formerly named Hashimoto's encephalopathy, may manifest acutely or insidiously with impairment of consciousness, frequently evolving into coma. The disease also occurs in children. Patients may show a variety of additional neurological and/or psychiatric symptoms. Seizures and SE complicate the course in about 70-90% of subjects (Castillo *et al*., 2006).

Rasmussen encephalitis is a rare and severe immune-mediated brain disorder that results in unilateral hemispheric atrophy with progressive neurological dysfunction and intractable seizures. Typically, seizures start during childhood, and difficult-to-treat focal motor seizures or epilepsia partialis continua are a constant feature of the disease. Hemispherectomy is still the only effective therapy, achieving seizure freedom in 62.5 to 85% of patients (Bien *et al*., 2005).

### Hemiconvulsion-hemiplegia-epilepsy syndrome (HHE) and febrile infection-related epilepsy syndrome (FIRES)

HHE and FIRES are rare childhood epilepsy syndromes that share an occurrence of refractory SE during or after fever, without evidence of central nervous system infection (Nabbout, 2013).

HHE is characterised by definitive hemiparesis following a prolonged unilateral febrile seizure in children usually before four years of age. Epilepsy, often of a complex-partial type, sets in a few months later, and cognitive sequelae are frequent. Longitudinal neuroimaging demonstrates unilateral cerebral swelling, increased signal intensity on T2 weighted images, and decreased water diffusion in DWI within the first two weeks after the febrile convulsion. Such alterations resolve by 3-4 weeks, when an extensive and progressive cortical and subcortical atrophy becomes obvious (Welcker *et al*., 2013).

FIRES is characterized by the development of seizures in healthy children during or a few days after a non-specific febrile infection. Seizures rapidly worsen and evolve into status epilepticus, followed by pharmacoresistant epilepsy and severe mental deficits (van Baalen *et al*., 2010).

The pathogenesis of HHE and FIRES has not been resolved, but it has been hypothesized that both entities share a common pathophysiology based on a vicious cycle in which inflammation-induced seizures evolve into status, which then enhances inflammatory pathways and ensures that they remain active (Nabbout *et al*., 2011; Nabbout, 2013).

### Recent genetic findings in epilepsies associated with prolonged seizures and SE

Whole-exome sequencing, single nucleotide array analysis, and gene panel diagnostics are new powerful techniques that have facilitated our understanding of the genetics of epilepsies manifesting in childhood (Lemke *et al*., 2013). Application of these methods has broadened the phenotypic spectrum of some epilepsy syndromes, and has identified new genes involved in their pathogenesis. *Table 1* summarizes some recently identified genes related to more severe forms of epilepsy. □

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# Syndromes with very low risk of acute prolonged seizures

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**ABSTRACT** – The provision of rescue medication is an important component in the treatment of epilepsy. An intervention within five to ten minutes in the case of an acute prolonged seizure may preserve the patient from status epilepticus (SE). However, the risk of convulsive SE (CSE) differs markedly between patients depending on individual factors. This report summarizes the literature on risk factors for CSE in children with epilepsy and adolescents, and discusses the hypothesis that some electroclinical syndromes engender a very low risk of CSE. The most important risk factor for SE is the history of a previous event. The longer a patient lives without SE, the lower the risk will be. CSE occurs significantly less frequently in idiopathic epilepsies compared to epilepsies with symptomatic or unknown aetiology. It is very rarely observed in patients with (non-encephalopathic) idiopathic generalised epilepsies, *i.e.* childhood absence epilepsy or juvenile myoclonic epilepsy. However, non-compliance or inappropriate treatment may trigger CSE in these syndromes. A very low risk can be assumed for children with Rolandic epilepsy, while CSE occurs in a considerable percentage of patients with Panayiotopoulos syndrome. Although the risk of CSE in otherwise normal children with cryptogenic focal epilepsy is uncertain, it is presumably low under successful continuous medication. In conclusion, the choice for or against the prescription of rescue medication remains an individual decision. Consequently, for several electroclinical syndromes, a *per se* provision of rescue medication does not appear justified.

**Key words:** childhood, status epilepticus, electroclinical syndrome, idiopathic epilepsy, rescue medication

After an initial unprovoked seizure, the incidences of acute prolonged seizures and status epilepticus (SE) are 47/100,000 children/year and 27/100,000 children/year, respectively (Eriksson *et al.*, 2005). More than 15% of children with epilepsy will experience at least one episode of SE (Fountain, 2000). Generalised tonic-clonic seizures (GTCS) are usually short in duration. In the case of a seizure lasting more than 10 minutes, there is a high risk of evolution leading to convulsive status epilepticus (CSE) (Lowenstein *et al.*, 1999; Eriksson *et al.*, 2005). SE is defined by a seizure duration of longer than 30 minutes and is associated with numerous risks, including mortality (Eriksson *et al.*, 2005). Acute prolonged seizures are typically not self-limited and require treatment. Most acute prolonged seizures do not occur in the hospital, but in different situations of daily life (Alldredge *et al.*, 2001). The incidence of CSE can be reduced by an effective

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treatment in the pre-hospital phase (Eriksson *et al*., 2005; Chin *et al*., 2008). Besides the benefit for the patient him/herself, the reduction of hospital admissions can help to save costs (Lee *et al*., 2013).

Although different forms of effective drugs are available, the question arises as to whether rescue medication should be prescribed to each and every patient with epilepsy. The risk of acute prolonged seizures and CSE varies between different electroclinical syndromes and depends on other individual factors. Many patients may not require a provision of rescue medication because of a very low risk of CSE. Major arguments for not prescribing rescue medication in these cases may be the necessity to reduce costs and the possible negative psychosocial effects for patients. The fact that a patient must carry rescue medication on his person on a daily basis serves to remind patients, and their parents, of the fact that they have epilepsy. The continuous concern may influence the interaction with caregivers, and the psychosocial development of patients may be impaired in cases with an otherwise excellent prognosis.

The epidemiological studies on SE and prognosis in childhood epilepsy typically do not report on acute prolonged seizures, since this is a more recently used term. Therefore, this review focuses on the risk factors for CSE. In summary, careful recommendations are given regarding individual constellations in which there seems to be no necessity of providing rescue medication to a child with epilepsy.

## Sources of evidence and their limitations

Prospective studies that include children with a first unprovoked seizure or new-onset epilepsy are the gold standard. They provide data on risk factors, type, frequency, timing, recurrence, and outcome of SE in patients with epilepsy (Wirrell *et al*., 1995; Wirrell *et al*., 1996; Shinnar *et al*., 1997; Shinnar *et al*., 2001; Sillanpää and Shinnar, 2002; Berg *et al*., 2004; Stroink *et al*., 2007; Callenbach *et al*., 2009; Camfield and Camfield, 2009; Wirrell *et al*., 2011; Camfield and Camfield, 2012). Prospective studies that include patients with a first episode of SE of any aetiology provide less convincing information regarding risk factors in patients with pre-existing epilepsy (DeLorenzo *et al*., 1995; Cascino *et al*., 1998; Chin *et al*., 2006; Hesdorffer *et al*., 2007). However, risk factors for recurrence and outcome can also be analysed appropriately using these types of studies. At a lower level of evidence, important information arises from retrospective studies that usually include hospital-based cohorts with SE or different types of epilepsy (Loiseau *et al*., 1983; Deonna *et al*., 1986; Loiseau *et al*., 1995; Ferrie *et al*., 1997; Novak *et al*., 1997; Caraballo *et al*., 2000; Genton *et al*., 2001; Larch *et al*., 2009).

Prospective studies on the prognosis of childhood epilepsies and SE frequently do not differentiate between various forms of SE, *i.e*. CSE, non-convulsive SE (NCSE), myoclonic SE (MSE), and others. Of even greater importance is the fact that electroclinical syndromes are usually not discriminated. There is a lack of information regarding the time of occurrence of SE in relation to antiepileptic treatment. SE may occur before initiation of treatment, under continuous treatment, when changing drugs, or after stopping the therapy. Information about non-compliance, inappropriate medication, or other trigger factors is almost always lacking. Finally, the studies do not provide any information on the usage of rescue medication which may theoretically lower the rate of SE in epilepsy patients. Some of the retrospective studies on patients with specific epileptic syndromes provide more details on these important factors. Therefore, they should be considered when trying to answer the question about the necessity of rescue medication. However, the variation in syndrome definitions constitutes a major shortcoming of all these studies. To give an example, Valentin *et al*. (2007) compared the application of the 1989 ILAE definition of childhood absence epilepsy with the 2005 ILAE Task Force proposal. The more strict criteria of the 2005 proposal allowed the classification of CAE for only 30 of 44 children who had been previously diagnosed with CAE. A difference is presumed regarding prognosis and the risk of generalised tonic-clonic seizures (GTCS).

## Epilepsy in patients with a first SE and SE in patients with epilepsy

About one third of patients who present with a first episode of SE have a history of previous unprovoked seizures or pre-existing epilepsy (Hauser *et al*., 1997). While Chin *et al*. (2006) found that 7.4% of patients under 15 years of age had an epilepsy diagnosis, this proportion was 38% in patients <16 years in the Richmond population (DeLorenzo *et al*., 1995). Of the patients with a first unprovoked SE, 25.5% were found to have epilepsy in the Rochester population (Cascino *et al*., 1998).

Fountain (2000) reported a risk of SE of at least 15%, in patients with epilepsy. In the Connecticut study, SE occurred in 9.1% of children before epilepsy was diagnosed (Berg *et al*., 1999). After the diagnosis was made, SE occurred in 9.5% (Berg *et al*., 2004). In patients from the Dutch population, the risk of SE was 9.5% within a five-year follow-up period (Stroink *et al*., 2007). A higher risk of 27% was reported for the Finish population (Sillanpää and Shinnar, 2002). Camfield and

Camfield reported at least one SE in 20% of otherwise normal children from Nova Scotia with focal epilepsies (2012).

SE is the initial event in 6.5-12% of children presenting with a first unprovoked seizure or a new-onset epilepsy (Shinnar *et al*., 1990; Hauser *et al*., 1997; Berg *et al*., 1999; Shinnar *et al*., 2001; Berg *et al*., 2004; Camfield and Camfield, 2012; Stroink *et al*., 2007). A recurrence of SE can be expected in about one third of the patients (Berg *et al*., 2004; Stroink *et al*., 2007).

## Risk factors for SE in children with epilepsy

The most important risk factor for SE is the presence of SE in the past (Shinnar *et al*., 1996; Novak *et al*., 1997; Hesdorffer *et al*., 1998; Shinnar *et al*., 2001; Sillanpää and Shinnar, 2002; Berg *et al*., 2004; Stroink *et al*., 2007). Berg *et al*. reported a cumulative probability of 32% for an episode of SE during eight years of follow-up in patients with a history of prior SE, compared to 7.2% in patients without (Berg *et al*., 2004). The duration of the second seizure was highly correlated with the duration of the initial event (Shinnar *et al*., 2001). Of 25 children who initially presented with SE for a duration of ≥30 minutes, the duration of the second seizure was ≥10 minutes in 44%, ≥20 minutes in 36%, and ≥30 minutes in 24%, respectively.

Other risk factors for SE comprise young age (Shinnar *et al*., 2001; Sillanpää and Shinnar, 2002; Berg *et al*., 2004), symptomatic aetiology (Shinnar *et al*., 1997; Berg *et al*., 2004; Chin *et al*., 2006; Stroink *et al*., 2007), non-idiopathic (cryptogenic) aetiology (Shinnar *et al*., 1997; Berg *et al*., 2004; Stroink *et al*., 2007), history of febrile convulsions (Sillanpää and Shinnar, 2002; Stroink *et al*., 2007), focal epilepsy (Berg *et al*., 1999; Sillanpää and Shinnar, 2002), and prior craniotomy (Berg *et al*., 1999). Although rarely mentioned, non-compliance is presumably another important risk factor (Fountain, 2000).

## Factors associated with a low risk of SE in epilepsy

The risk of SE is low in patients presenting with brief seizures at epilepsy onset (Shinnar, 2007). In 137 cases with an initial seizure duration ≤10 minutes, the second seizure lasted ≥10 minutes in 8%, ≥20 minutes in 4%, and ≥30 minutes in only 1% (Shinnar *et al*., 2001). In the event that the first two years pass without SE, the risk of SE decreases markedly (Sillanpää and Shinnar, 2002; Hesdorffer *et al*., 2007).

The relationship with antiepileptic treatment is another important factor. Stroink *et al*. (2007) observed a recurrence of SE in 14 of 44 children (34%) within five years of follow-up. However, SE occurred in only 3 patients when the antiepileptic medication was continued unchanged. The antiepileptic medication had not yet been started or had already been stopped in 11 patients at the time of SE.

The risk of SE is much lower in patients with idiopathic epilepsies compared to other aetiologies. In the Dutch study, SE during the five-year follow-up period was observed only in patients with idiopathic epilepsy who had already experienced a SE as the initial event (Stroink *et al*., 2007). In this cohort, the lowest rate of SE was found in patients with idiopathic epilepsy and without a history of febrile seizures. Comparably, the rate of SE in the Connecticut cohort was the lowest in patients with idiopathic epilepsy (4%) (Berg *et al*., 2004).

A lower risk of recurrence in idiopathic cases was also found in studies on outcome after a first SE. A recurrence of SE was noted in only 4% of cases with idiopathic aetiology compared to 44% with remote symptomatic and 67% with progressive aetiology (Shinnar *et al*., 1997).

## CSE in patients with (non-encephalopathic) idiopathic generalised epilepsy (IGE)

Shorvon and Walker reviewed the available data on this topic (2005) and stated: "Convulsive SE is surprisingly uncommon in IGE and much less common than in the secondary generalised or partial epilepsies. This point has been long established, although the older studies, which have given frequency figures, have generally failed to differentiate idiopathic and cryptogenic generalised epilepsies. The true frequency in IGE is, therefore, unclear; however, clinical experience suggests that tonic-clonic status is rare in IGE and also, when it does occur, there is usually a complicating factor such as drug withdrawal".

In a prospective cohort of patients presenting with new-onset epilepsy, 5.9% of the 136 children and adolescents with IGE experienced at least one SE (Berg *et al*., 2004). The rate was 3.9% in 113 cases without initial SE. In the Dutch study, 3% of 204 children with IGE presented with SE at onset, but none of the remaining 198 patients experienced SE during the five years of follow-up (Stroink *et al*., 2007). Therefore, the risk of SE appears to be low in IGE without initial presentation of SE.

In children with childhood absence epilepsy (CAE), the rate of patients with GTCS varies between 8 and 25% (Loiseau *et al*., 1983; Loiseau *et al*., 1995; Wirrell *et al*., 1996; Callenbach *et al*., 2009). In the Nova Scotia cohort, 9.7% of 72 children with CAE presented with

GTCS, and 4 experienced absence SE while on medication (Wirrell *et al*., 1996). In contrast, this type of status occurred late in the course and after stopping medication in 5 of 6 patients with GTCS in the Dutch study (Callenbach *et al*., 2009). Loiseau *et al*. (1983) also found that most GTCS in CAE occurred after 5 to 10 years, and the rate was 36% in 90 patients. In another cohort, Loiseau *et al*. (1995) found a difference in risk for GTCS depending on the age at onset of CAE. While the percentage of patients with GTCS was 16.2% in the case of onset under the age of 9 years, it increased to 43.7% in children with onset at 9 or 10 years of age. The total rate of GTCS was 25% in 52 patients. The rate of GTCS is much higher in patients with juvenile absence epilepsy (JAE). GTCS occurred in 79% of 62 patients in a study by Loiseau *et al*. (1995). Two of these patients experienced absence SE. Trinka *et al*. (2004) differentiated pyknoleptic (*n*=81) *vs*. non-pyknoleptic (*n*=82) absences and found rates of GTCS of 8.5 and 7.4%, respectively. Unfortunately, none of the studies provides information on the duration of GTCS in absence epilepsies. However, although absence SE may complicate CAE, no cases of CSE have been reported in these studies.

Camfield and Camfield (2009) analysed the long-term outcome in 24 patients with juvenile myoclonic epilepsy (JME) from Nova Scotia. All patients had at least one GTCS, since this was an inclusion criterion. The authors stated that at least 90% of patients with JME suffered from GTCS. Eight patients (3%) from this cohort presented with at least one episode of CSE during the 25-year follow-up period. Other studies did not report any cases with CSE with regards to generalised tonic-clonic seizures. Larch *et al*. (2009) noticed myoclonic SE (MSE) in 3% of 247 patients. The rate of patients with NCSE was 5.8 to 6.7% in other reports (Agathonikou *et al*., 1998; Dziewas *et al*., 2002). There is a belief that in JME, SE of any form is clearly associated with sleep deprivation, AED withdrawal, or inappropriate treatment (Genton *et al*., 2000; Shorvon and Walker, 2005; Baykan *et al*., 2013; Crespel *et al*., 2013). Inappropriate treatment with carbamazepine, phenytoin, vigabatrin, or gabapentin can be the cause of SE in different forms of IGE (Panayiotopoulos *et al*., 1997; Genton *et al*., 2000; Dziewas *et al*., 2002; Thomas *et al*., 2006; Larch *et al*., 2009).

## CSE in patients with idiopathic focal epilepsy

Deonna *et al*. (1986) reported on 107 neurologically normal children with partial epilepsy who were included in a retrospective, hospital-based study. Benign focal epilepsy was only diagnosed in patients with simple partial seizures that clinically presented with a Sylvian onset (or component) or sensorimotor symptoms (*n*=38). Patients with complex partial seizures were subsumed in a different group (*n*=31). In 4 patients, the diagnosis of benign focal epilepsy was based only on the clinical symptoms, since the waking EEG showed non-specific pathology and no sleep recordings were available. Severe seizures were defined as SE and prolonged seizures with a duration >15 minutes or clusters, and these occurred in 24% of the 38 children, including 11% who presented with SE. The authors stated that, despite therapy, severe seizures occurred in one third of the patients. It remained unclear whether severe seizures were focal or secondary generalised, and whether the 4 patients with only non-specific EEG changes were affected. However, the general prognosis in this group was excellent for all patients, and only one child continued to have seizures after the age of 15 years. Berg *et al*. (2004) could not identify even a single patient with SE out of a group of 66 children with idiopathic partial epilepsy. The results from the Dutch cohort were similar, with no case of SE reported in 30 children with idiopathic localisation-related epilepsy (Stroink *et al*., 2007). SE occurred in 3 of 42 children with Rolandic epilepsy from the Nova Scotia cohort (Wirrell *et al*., 1995). However, atypical features were present in 2 of the children. Developmental delay was found in one child; the other presented with an abnormal EEG background activity and seizures which occurred only during the daytime. No information was provided regarding the relationship to time of diagnosis and antiepileptic treatment. The same group reported another study of 79 patients with Rolandic epilepsy (Peters *et al*., 2001). Whereas 36 patients (46%) were not treated with antiepileptic drugs at any time, 43 (54%) were treated with antiepileptic drugs. The initial therapy was comprised of carbamazepine in 82%, phenobarbital in 12%, and clobazam in 7%. The treatment was changed in 28% of the patients. The rate of GTCS was 50% in the group without treatment, compared to 16% in treated patients. CSE was observed in only one case that was not treated with antiepileptic drugs. However, the long-term prognosis was excellent in both groups as well as in the patient with SE. In summary, CSE is a rare event in patients with classic Rolandic epilepsy. This appears to be particularly true for children treated with antiepileptic drugs.

The situation is completely different for children with Panayiotopoulos syndrome (PS), for whom the risk of CSE is high. In 113 children recruited from multiple centres, partial SE occurred in 44% (Ferrie *et al*., 1997). An evolution with secondary generalisation (CSE) was observed in 16%. A prospective study from Argentina showed similar results (Caraballo *et al*., 2000); 20 of 66 patients presented with partial SE, which led to secondary generalisation and evolved to CSE in 25%.

## SE in cryptogenic focal epilepsy

Wirrell *et al*. (2011) compared the outcome of 111 children with cryptogenic partial epilepsy (normal cognition in 66%, mildly delayed in 24%, and severely delayed in 10%) with 95 patients suffering from symptomatic partial epilepsy. Of the children with cryptogenic partial epilepsy, 14% initially presented with SE. After diagnosis, a first SE was observed in only 3.1% of the remaining patients. In contrast, 28% of the children with symptomatic partial epilepsy initially presented with SE, and a first SE during follow-up occurred in 43% of the remaining patients.

Camfield and Camfield (2012) identified 188 children under the age of 16 years with both normal IQ and neurological status, including 23 patients with Rolandic epilepsy. SE occurred in 39 patients, of whom 19 presented with an initial SE at the time of diagnosis. Twelve patients experienced multiple (2 to 10) episodes with SE. Of the 39 otherwise normal children with complex partial seizures reported by Deonna *et al*. (1986), 46% suffered from "severe seizures" (8% SE, 8% clusters, and 33% prolonged seizures >15 minutes). The rate of "severe seizures" in 36% was not significantly different in children with non-Rolandic simple partial seizures (12% SE, 16% clusters, and 16% prolonged seizures). However, the aetiologies in otherwise normal children with partial epilepsy were not reported for these studies and patients with structural lesions may have been included.

Stroink *et al*. (2007) identified initial presentation with SE in 15/70 patients (21.4%) with cryptogenic partial epilepsy in the Dutch study. A first SE occurred in only 2 of the remaining 55 children after a diagnosis of epilepsy had been established (3.6%). Berg *et al*. (2004) reported an initial SE in 7.7% of 221 children with cryptogenic partial epilepsy, and the first SE followed diagnosis in 7% of the remaining 199 cases.

Altogether, the incidence of SE in patients with cryptogenic partial epilepsy, or otherwise normal children with partial epilepsy, remains unclear.

## Conclusion

The question as to whether or not rescue medication should be prescribed for an individual patient depends on many factors. The risk of experiencing acute prolonged seizures and SE obviously influences this decision. This risk is low in children and adolescents with appropriately treated CAE or JME. Patients with classic Rolandic epilepsy may not need rescue medication. The risk of SE is uncertain in otherwise normal children with partial epilepsy of unknown aetiology. However, it seems to be low in children who are seizure-free under continuous antiepileptic treatment and who have no history of previous SE.

Since even the rare event of a CSE may harm the individual patient, it is not possible to give a general recommendation for or against rescue medication based only on the syndrome diagnosis. The given infrastructure, such as the availability of ambulant emergency care and the distance to the next emergency room, is of great importance. The number of involved caregivers (for example, teachers) and their role in daily life may be important, since all of them should be advised on when and how to administer rescue medication to an individual child. Most importantly, the willingness of the properly-informed family itself is critical. Rescue medication may even be prescribed to a child with a very low risk of CSE. On the other hand, it may not be prescribed to another child with a comparably higher risk, whose parents may be less anxious and live close to the nearest emergency facility.

The most important risk factor for SE is the history of a previous event. Therefore, rescue medication is recommended for this subgroup of patients independent of the epilepsy syndrome.

Based on the available data, the following statements can be made:

– the risk of SE is low in patients who experienced initially brief seizures;

– most episodes of SE occur at epilepsy onset and within the first two years after diagnosis;

– the longer a patient lives without SE, the lower is the risk of experiencing SE;

– the risk of SE is low with continuous, appropriate and successful medication;

– the risk of SE is low in idiopathic generalised epilepsies. In appropriately-treated CAE and JME, the risk is very low;

– the risk of harmful CSE is very low in Rolandic epilepsy. In contrast, Panayiotopoulos syndrome frequently presents with CSE;

– the risk of CSE is uncertain in patients with cryptogenic partial epilepsies. However, it is much lower when compared in patients with symptomatic partial epilepsy. □

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# Is there a need for further trials for the treatment of prolonged seizures?

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**ABSTRACT** – Prolonged seizures are associated with morbidity and mortality of varying degrees. It is important to recognize seizures early, and treat them appropriately. This leads to the best clinical outcome. There has been an emphasis on prompt treatment, but there exists a variety of poorly executed protocols. This review addresses the question of whether additional clinical trials are necessary, not only to answer for what purpose, but also, clearly, to examine the impact additional studies may have. Overall, the acute treatment of epilepsy emergencies in children has markedly improved with availability of out-of-hospital therapies, but additional studies to determine the most efficacious, maximally safe, and best tolerated treatments are needed.

**Key words:** status epilepticus, prolonged seizure, acute seizure, treatment, trial, benzodiazepine

Children with seizures experience a spectrum of events extending from isolated, brief seizures to status epilepticus (SE), incorporating a full range of recurrent unprovoked seizures and prolonged or acute repetitive seizures. Similarly, semiology varies from partial to generalised, and non-convulsive to violent and continuing convulsions. This symposium has clearly discussed the early recognition and treatment of repetitive and prolonged seizures because of the deleterious effect with various degrees of morbidity and mortality. Prompt recognition and management leads to the best chance of successful outcome. Further treatment paradigms, appropriate for use in out-of-hospital settings and in hospital, are emphasized and recommended. These protocols must be well designed, and if appropriate, can be followed by the inexperienced and experienced, either caregivers or medical professionals. Even with emphasis on prompt treatment over the past twenty years, there exists a variety of poorly executed protocols. This review addresses the question of whether additional clinical trials are necessary, not only to answer for what purpose, but also, clearly, to examine the impact additional studies may have.

The current definition of SE has shortened from a 30-minute duration of continual recurrent seizures without recovery (ILAE, 1981) to the "operational definition of five minutes or more of continuous seizures or two discrete seizures between

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which there is incomplete recovery of consciousness" (Lowenstein *et al.*, 1999). The latter definition and aggressive early treatment, certainly justified by experimental and clinical data, have demonstrated a tenfold lower rate of mortality for patients with seizures lasting less than 30 minutes. Furthermore, ample data suggests much improved response to first-line therapies if treated earlier. The definition of SE in neonates, however, is even more controversial, as not only timing is argued, SE has so far only been described according to electroclinical, electrographic and clinical features (Morton and Pellock, 2012).

Multiple authors have noted that the outcome of status epilepticus and prolonged or recurrent seizures is dependent upon age, aetiology, and duration of events. Infants younger than one year of age represent a subgroup of children with the highest incidence of SE, whether events, total incidents or recurrences are counted (DeLorenzo *et al.*, 1996; Logroscino *et al.*, 1997). Similarly, causes associated with SE in children are age dependent. Shinnar *et al*. (1997) reported that more than 80% of children younger than 2 years of age had SE resulting from a febrile or acute symptomatic cause. Cryptogenic or remote symptomatic causes are more common in older children. In adults, sub-therapeutic levels of antiepileptic drugs, remote causes, and cerebrovascular disease were the three most common causes of SE (DeLorenzo *et al.*, 1995). Thus, the situation of a child developing SE will differ from neonates to adolescents. For any trial of treatment, appropriate dosing, route of administration, along with time to treatment and definition of repetitive (or clusters of) seizures and SE should be considered. Although it is difficult to correlate treatment with aetiology, large studies may demonstrate differences.

## Management and therapy

SE and acute repetitive seizures (clusters) represent neurological medical emergencies. The goals of treatment are noted in *box 1*.

Most prolonged seizures occur out of hospital. Following initial emergent and supportive care, as well as treatment of potential hypoglycaemia, benzodiazepine administration represents the most favoured and evidence-based acute treatment for seizures. Various routes of administration exist, but intravenous is recommended when readily available. Following one or two doses of benzodiazepine, various protocols have been designed and reviewed, with some organizations proposing guidelines, such as the Neurocritical Care Society and American Epilepsy Society. Following benzodiazepine administration, fosphenytoin/phenytoin and valproate are frequently recommended, with some centres continuing the use of phenobarbital. Because of the complexity of studies and clinician bias, there is still argument regarding the best first and second-line therapy. However, most would agree that although a benzodiazepine is the preferred first-line intervention (Rossetti and Lowenstein, 2011). Thus, the primary inquiry may concern which benzodiazepine to use for first-line treatment.

**Box 1.**
**Management Goals for Acute Therapy of Prolonged and Repetitive Seizures**

Ensure adequate brain oxygenation and cardiorespiratory function
- Terminate clinical and electrical seizure activity as rapidly as possible
- Prevent seizure recurrence
- Identify precipitating factors, such as hypoglycaemia, electrolyte imbalance, lower drug levels, infection, and fever
- Correct metabolic imbalance
- Prevent systemic complications

Before discussing whether trials could possibly determine which benzodiazepine treatment is superior, a brief review of second-line therapy and trials will be discussed. During the past two decades, protocols were developed in both Europe and the United States which delineated early, established and refractory stages of SE and treatment algorithms. The Veterans Administration study (Treiman *et al.*, 1998) compared combined diazepam and phenytoin, phenytoin alone, phenobarbital, and lorazepam for the treatment of SE and established the more rapid efficacy of benzodiazepine. Subsequently, protocols for refractory SE have been developed and are generally accepted, and have been modified and adopted by different groups (Rosetti and Lowenstein, 2011; Riviello *et al.*, 2013). Second-line preference for phenytoin/fosphenytoin continues, but others have suggested valproate or phenobarbital as nearly equivalent, especially for the treatment of children. Third-line treatment includes midozalam, propofol, and levetiracetam. Pentobarbital, propofol, and anaesthetic agents are typically reserved for refractory or super-refractory cases (Shorvon and Ferlisi, 2011; Rossetti and Lowenstein, 2011). Multi-centre, randomised, double-blind trials were designed to determine the most effective and/or least effective treatments of established status epilepticus in a patient older than the age of 2 years, as opposed to comparing fosphenytoin, levetiracetam, and valproate. The primary outcome measure was cessation of clinical seizure activity and improvement of mental status without serious adverse effects or further intervention at 60 minutes after administration of study drug (Bleck *et al.*, 2013).

## Benzodiazepine

Currently in the United States, the only FDA-approved drug recommended for treatment of break-through seizures is rectal diazepam gel. The National Institute of Clinical Excellence (NICE) in the UK recommends rectal diazepam with buccal diazepam for out-of-hospital initial therapy for prolonged seizures in children (NICE, 2012). In other countries, additional preparations are licensed. Furthermore, a number of unlicensed methods for administrating benzodiazepines have been popularized and are currently in use worldwide. Diazepam, alprazolam, clobazam, clonazepam, lorazepam, and midazolam have been used through intravenous, oral, intramuscular, buccal, nasal, and rectal routes. Thus, there is a need for further studies evaluating initial treatment of seizure emergencies. Although there is also a need for paediatric studies of other antiepileptic drugs, this discussion will focus on first-line treatment of paediatric seizures.

### Paediatric study variables

Pre-hospital treatment with multiple benzodiazepine preparations has been demonstrated to reduce seizure activity significantly, compared with seizures that remain untreated until the patient reaches the emergency department. The optimal agent for treatment of paediatric seizure emergencies remains unclear, although a recent article concluded that intravenous lorazepam is the expert consensus for first-line treatment of prolonged seizures in children (Riviello *et al.,* 2013). Another suggests that intramuscular midazolam is superior (Silbergleit *et al.,* 2013). Studies that specifically evaluate the paediatric population are limited, and the age range of children recruited varies. Studies have recruited patients as young as 1 month of age (Fişgin *et al.,* 2002) or less than 18 years old, as stipulated as a study criterion (Holsti *et al.,* 2010). The recruitment age of patients in paediatric studies has a larger role than in adult studies because of medication dosing. Children are dosed with benzodiazepines based on weight and age (Diastat$^{R}$) and if the study does not account for both of these factors, the results are affected.

Location of recruitment will also affect the patient population. For example, studies that are performed in locations such as Sub Saharan Africa will have different aetiologies of prolonged seizures because of the high incidence of cerebral malaria (Malu *et al.,* 2013). The aetiology of the seizures may affect the outcome and response of patients recruited, and makes it difficult to compare studies from different regions. The availability of medications will also be based on the region where the study is performed. For example, intravenous lorazepam is not available in France and clonazepam is used as a first-line benzodiazepine (Hubert *et al.,* 2009), which is not available in the United States.

Inclusion criteria and outcome vary between studies. Seizure emergencies include acute seizure management of repetitive seizures, prolonged seizures, and SE. The inclusion criteria definition can vary for each of these situations and the possibilities are too numerous to enumerate. There are primary outcome and secondary outcome measures that include time to treatment, superiority of route of administration or drug, seizure cessation, and seizure recurrence. The different outcomes make comparison and analysis of multiple studies challenging.

### Medication formulation

There are numerous studies that compare the different benzodiazepines as abortive treatment in children, but the formulation of the medications used differs even when similar routes are used. Midazolam is an example of a medication that has been studied as an intranasal medication option, but in some studies the available intravenous formulation was dripped into the nose (Lahat *et al.,* 2000; Mahmoudian and Zadeh, 2004; Bhattacharyya *et al.,* 2006; Javadzadeh *et al.,* 2012; Thakker and Shanbag, 2013), while in others it was sprayed into the nares (Fişgin *et al.,* 2002; Holsti *et al.,* 2010). Buccal midazolam formulations include maleate and the classic intravenous preparation. This can affect the efficacy and adversity of the treatment because different delivery preparations and systems are difficult to compare. Nevertheless, efficacy is reported.

### Routes of administration

It is difficult to compare studies when there are different routes of medication administration, especially when two different medications are compared. It is well accepted that the time required to administer an intravenous preparation in a convulsing patient can delay treatment and prolong seizure duration. For example, studies have concluded that seizure cessation was faster with diazepam, but the time to administer intravenous diazepam was greater than that for intranasal midazolam (Lahat *et al.,* 2000; Mahmoudian and Zadeh, 2004; Javadzadeh *et al.,* 2012). Time and ability to establish intravenous access has led some to interosseous administration. The overall treatment effect (time to administer treatment *and* achieve seizure cessation) was significantly improved with intranasal midazolam when compared with intravenous diazepam (Mahmoudian and Zadeh, 2004; Bhattacharyya *et al.,* 2006; Javadzadeh *et al.,* 2012; Thakker and Shanbag, 2013). Comparing rectal, intranasal, and intravenous formulations can cause

confusion because of the factors that affect time to administration.

The RAMPART trial (Rapid Anticonvulsant Medication Prior to Arrival Trial) reported a double-blind randomised clinical trial to determine if the efficacy of intramuscular midazolam is non-inferior by a margin of 10% to that of intravenous lorazepam in patients treated by paramedics for SE (Silbergleit *et al.,* 2013). SE was defined as the patient actively convulsing upon EMS arrival. The study concluded that patients treated with intramuscular midazolam were more likely to have stopped seizing on arrival to the emergency department and were less likely to require any hospitalisation or admission to an intensive care unit. This was a large study, included adults and paediatric patients, and concluded that intramuscular midazolam is not less effective, but not superior. Further review of the results of the study support that "*intramuscular midazolam is the best option for the prehospital treatment of status epilepticus*" (Silbergleit *et al.,* 2013).

A meta-analysis was published in 2010 to evaluate midazolam versus diazepam in children and young adults (McMullan *et al.,* 2010). The study concluded that for seizure cessation, midazolam by any route was superior to diazepam, non-intravenous midazolam was as effective as intravenous diazepam, and buccal midazolam was superior to rectal diazepam with regards to achieving seizure control. The limitation of this meta-analysis is that all studies included were relatively small and were not standardised relative to dose, outcomes, and inclusion criteria. The results support the conclusion that more larger and standardised studies are needed to determine superiority of a benzodiazepine.

## Discussion and conclusion

Cross *et al*. (2013) recently published an intriguing paper entitled "*Are we failing to provide adequate rescue medication to children at risk of prolonged convulsive seizures in schools?*" They highlighted current guidelines recommending immediate treatment of children to prevent progression to status epilepticus and emphasized that a more systematic response is needed to ensure that children receive rescue medication regardless of where their seizure occurs. In the United States, local regulations may allow or disallow administration of benzodiazepine. Certain cultures will find it socially difficult to administer the rectal formulation in all but the youngest of children. Furthermore, international agreement and availability of medication differs. In 2009, Hubert reported that intravenous lorazepam was not available in France. Furthermore, buccal midazolam is not licensed in the United States. Their suggestion of using intravenous clonazepam may be appropriate in some countries, but this preparation is not available in the United States.

With this degree of practical concern and controversy, the academic scientist/clinician must certainly request additional studies to answer which agent is the most effective and safe for the management of acute seizures in children. In the UK, Chin reported that only one in every six children with SE admitted to the paediatric intensive care unit was appropriately treated using existing current guidelines. In the United States, the Febrile Status Epilepticus Study (FEBSTAT) demonstrated significant diversity, not only in home therapy for those with recurrent events, but in emergency services for initial treatment of SE. Furthermore, inadequate dosing was administered in a third of patients (Seinfeld *et al*., 2014). Similar disparities are noted across the UK and Europe for the administration of rescue medications in children with prolonged acute convulsive seizures in the community (Wait *et al.,* 2013).

## Further trials on treatment of acute seizures are needed

A large study to determine paediatric guidelines or algorithm for the treatment of seizure clusters (ARS) and prolonged seizures should be performed. Although it is accepted that treatment with benzodiazepine is first-line and should not be delayed, there is a lack of data to determine optimal benzodiazepine route, dose, or preparation.

There are fewer studies performed on acute paediatric seizures compared to those in adults. Larger paediatric studies are needed in paediatric seizure emergencies to determine first-line treatment and subsequent treatment. There is mounting evidence that supports multiple safe and effective alternative routes of benzodiazepine administration for rapid treatment of seizure emergencies in children. These studies must carefully define definition for treatments, appropriate dosing, age and aetiology of subjects, clearer outcome criteria (clinical, electrographic, or both), tolerability, and safety. Our belief is that there may be no single agent or method of administration optimal for all patients. Different age groups and personal preference of patients or caregivers will determine the "best" preparation for an individual. Nevertheless, studies will tell us if various compounds are comparable with regards to their efficacy and/or time to effectiveness. Certainly other factors will be important.

So, do we need further studies? YES!

Will this be easy to accomplish? No!

Our advice is to proceed with caution using large consortia with well-defined study criteria and end points. We all strive to provide the best and most efficient

treatment of potential seizure emergencies in children. Perhaps the greatest need for studies is to establish the acceptance of these therapies among those treating children. These investigations require the interaction of social scientists with medical professionals and lay organisations to establish best practices.
Overall, the acute treatment of epilepsy emergencies in children has markedly improved with availability of out-of-hospital therapies. Additional studies to determine the most efficacious, maximally safe, and best tolerated treatments are needed. We must also learn from those receiving and administering these treatments to optimize treatment for children of various ages with differing requirements. Still, we have unmet needs. □

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