Title: Why won't it stop? The dynamics of benzodiazepine-resistance in status epilepticus

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Abstract

Status epilepticus is a life-threatening neurological emergency that afflicts both adults and children. Approximately 40% of status epilepticus episodes do not respond to the current preferred first-line treatment, benzodiazepines, with a higher proportion of benzodiazepine refractoriness in resource-limited countries. These global differences in the response to benzodiazepines may, in part, be explained by the duration of status epilepticus at the time of treatment initiation. Evidence suggests that longer episodes of status epilepticus alter brain physiology in distinct phases, thus contributing to the emergence of benzodiazepine resistance. Such changes include alterations in GABA-A receptor function and in the transmembrane gradient for chloride, both of which erode the ability of benzodiazepines to enhance inhibitory synaptic signalling. Often, current management guidelines for status epilepticus do not account for these duration-related changes in pathophysiology. This may differentially impact resource-limited settings, where the time to reaching medical attention is longer on average. An updated understanding of pathological mechanisms in status epilepticus may enable reappraisal of known effective treatments such as phenobarbital. In this review, we aim to combine clinical insights and the latest evidence from basic science to inspire a new, context-specific approach to efficiently managing status epilepticus.

Key points

- The duration of seizure activity can alter the efficacy of benzodiazepines as a first-line treatment in convulsive status epilepticus
- Resource-limited countries have higher rates of benzodiazepine resistance in status epilepticus, likely due to delayed access to care
- Benzodiazepine resistance is contributed to by alteration in the structure and function of GABA-A receptors, which emerges over time following periods of extended seizure activity
- Changes to neuronal chloride regulation during status epilepticus can promote an excitatory shift in GABAergic signalling, which may diminish benzodiazepines efficacy
- Spatial differences in GABA-A receptor function across brain regions may account for differential responses to benzodiazepines
- Further research is needed into alternative first-line management strategies for people presenting with prolonged status epilepticus

Introduction

Epilepsy is a condition characterised by recurrent spontaneous seizures. It affects over 50 million people worldwide, the majority of whom live in resource-limited settings[1]. Seizures are associated with multiple risks, including fractures and bruising from injuries, head trauma and premature mortality. One of the most important causes of epilepsy-related mortality is convulsive status epilepticus (CSE), a state of unrelenting seizure activity that persists for more than five minutes[2]. CSE is a neurological emergency and prompt action to stop these prolonged seizures can reduce both morbidity and mortality.

Benzodiazepines (BZPs) can terminate seizures by enhancing $GABA_A$ receptor ($GABA_AR$) mediated signalling and are the preferred first-line management in CSE for both adults and children[3–7]. BZPs are easy to administer, cost-effective, and often successful in terminating SE, especially if used early following the onset of seizure activity[5, 7]. Failure of two adequate doses of appropriate BZPs to terminate SE necessitates second-line anti-seizure medications (ASMs) such as fosphenytoin, phenytoin, phenobarbital, levetiracetam, and valproate[8–11]. In some cases of SE, third-line management is required with anaesthetics such as thiopentone and propofol[12, 13]. BZP-resistant SE requires additional medications, sophisticated drug administration (including requiring syringe drivers for infusions and non-glucose prepared solutions for drugs such as phenytoin) as well as access to intensive care services that can provide close monitoring and invasive ventilation[5, 14]. These interventions may not be readily accessible, particularly to those living in resource-limited countries[15–19].

In this review, we explore BZP-resistant SE from both scientific and clinical perspectives. We focus on the more common convulsive SE (CSE) in adults and children and briefly discuss implications for other forms of SE, namely non-convulsive SE (NCSE) and neonatal SE. We review the current clinical literature to assess global trends in BZP-resistant CSE and discuss experimental research describing the possible pathophysiology underlying BZP resistance in patients. Here, we focus on the $GABA_AR$ - the principal target of BZPs - and explore the multiple seizure-induced changes that alter the sensitivity of $GABA_AR$ to BZPs during the evolution of ongoing seizure activity. Finally, we highlight unanswered questions and suggest possible considerations for improved treatment strategies based on the latest experimental studies and multicentre randomised clinical trials.

The global relevance of BZP-resistant CSE

It is difficult to accurately estimate the current epidemiology of SE owing to the overall significant differences in study designs and the relatively recent introduction of new diagnostic criteria, impeding comparability with studies conducted in the past decades[20–23]. Data are often not stratified across age groups or across different SE types, which makes it challenging to estimate the burden of CSE in adults and children. Based on available data, however, the global annual incidence of SE has been reported to range between 14 - 35/100 000 for children[24–26] and between 5 – 36/100 000 for adults[27–29]. As CSE is the most common presentation of SE, these figures might more closely reflect the incidence of CSE, potentially underestimating NCSE. There appears to be a bimodal age distribution with a peak incidence in early childhood and a progressive rise of SE incidence in the elderly[27, 30]. Febrile illness in children and stroke in adults are the most common causes of CSE[27, 28, 30–32]. In resource-limited countries, infectious causes such as cerebral malaria can add to the prevalence and severity of CSE [25, 33, 34].

The management of CSE has been of significant global academic interest for many decades, with the use and efficacy of BZPs being among the most studied topics[13]. A number of studies give either direct or indirect indication of the efficacy of first-line BZP monotherapy (**Table 1**). Globally, resistance to first-line treatment with BZPs occurs in

approximately 40% of patients (**Fig.1A**). Reported resistance is higher in studies from resource-limited countries. The large range in reported values (3-89%) likely reflects the significant heterogeneity between study designs and protocols. Our recent estimate of BZP resistance is more than double that quoted previously by Treiman in 1989[35] (~17% vs ~40%). This is most likely due to (i) the recent change in definition of CSE, (ii) the large number of studies conducted since that original report and (iii) the availability of more data from resource-limited countries.

The duration of CSE is an important indicator of whether a patient will respond to first-line BZPs[14, 36, 37]. Obtaining an accurate estimate of CSE latency, defined as the start of CSE to the time the first dose of BZP is given, is often difficult to determine as it relies on a witness being present when the CSE started or for care providers to record the time of seizure onset[23]. Also, the initial presentation may be of intermittent seizures that only later progress into CSE with the two being viewed as separate phenomena instead of as part of the same event. From the studies that do report CSE latency, however, it is clear that as the duration of CSE increases, so too does resistance to first-line BZPs (**Fig.1B**). In studies reporting CSE episodes exceeding 60 minutes, the resistance to first-line BZPs is as high as 53%. This phenomenon is likely to be more pronounced in resource-limited countries owing to challenges in healthcare access. Another important variable may be the underlying aetiology of CSE, but the current body of literature does not necessarily separate cases of BZP-resistant SE by cause of seizures. To gain an understanding of how underlying aetiology contributes to BZP resistance, further studies will be required.

Table 1: Studies reporting resistance to first-line treatment with BZP monotherapy in convulsive status epilepticus

Study	Country	Income Group[$]	Episodes*	Cohort	BZP-R	Latency
Das et al (2020)[38]	India	LMIC	94	Paediatric	89%	>60min
Burman et al (2019)[8]	South Africa	LMIC	144	Paediatric	48%	31-60min
Theusinger et al (2019)[39#]	Switzerland	HIC	126	Adult	28%	10-30min
Theusinger et al (2019)[39#]	Switzerland	HIC	39	Paediatric	3%	10-30min
Kay et al (2019)[40]	Germany	HIC	42	Adult	43%	31-60min
Hassan et al (2016)[41]	India	LMIC	84	Mixed	78%	>60min
Navarro et al (2016)[42]	France	HIC	68	Adult	16%	>60min
Chamberlain et al (2014)[43]	USA	HIC	273	Paediatric	15%	Not reported
Thakker et al (2013)[44]	India	LMIC	50	Paediatric	54%	31-60min
Silbergleit et al (2012)[45]	USA	HIC	509	Both	43%	Not reported
Mirsa et al (2012)[46]	India	LMIC	79	Adult	24%	10-30min
Gathwala et al (2012)[47]	India	LMIC	120	Paediatric	14%	Not reported
Arya et al (2011)[48]	India	LMIC	141	Paediatric	18%	Not reported
Chen et al (2011)[49]	China	LMIC	121	Adult	38%	31-60min
Skinner et al (2010)[50]	Honduras	LMIC	31	Adult	65%	>60min
Amare et al (2008)[51]	Ethiopia	LMIC	119	Adult	63%	31-60min
Chin et al (2008)[52]	UK	HIC	240	Paediatric	35%	31-60min
Mpimbaza et al (2008)[53]	Uganda	LMIC	330	Paediatric	37%	>60min
Ahmad et al (2006)[54]	Malawi	LMIC	80	Paediatric	25%	>60min
McIntyre et al (2005)[55]	UK	HIC	219	Paediatric	58%	31-60min
Qureshi et al (2002)[56]	UK	HIC	48	Paediatric	25%	31-60min
Fişgin et al (2002)[57]	Turkey	LMIC	45	Paediatric	42%	>60min
Mayer et al (2002)[58]	USA	HIC	83	Adult	69%	>60min
Alldredge et al (2001)[59]	USA	HIC	134	Adult	49%	31-60min

Tabarki et al (2001)[60]	Tunisia	LMIC	139	Paediatric	45%	>60min
Lahat et al (2000)[61]	Israel	HIC	44	Paediatric	5%	10-30min
Coeytaux et al (2000)[62]	Switzerland	HIC	172	Both	50%	31-60min
Scott et al (1999)[63]	UK	HIC	42	Both	33%	31-60min
Treiman et al (1998)[64]	USA	HIC	384	Adult	35%	31-60min
Chamberlain et al (1997)[65]	USA	HIC	24	Paediatric	8%	31-60min
Appleton et al (1995)[66]	UK	HIC	86	Paediatric	21%	31-60min
Remy et al (1992)[67]	France	HIC	39	Adult	28%	Not reported

[$]Income, classification based on gross national income (GNI) per capita (in US $) from the latest ratings[68]. Lower income countries, lower-middle income countries and upper-middle income countries are labelled as low-middle income countries and abbreviated to LMIC. High-income countries are abbreviated to HIC. *Episodes, refers to the number of episodes of CSE analysed in each study (sample size). [#]Data from same study across different age groups.

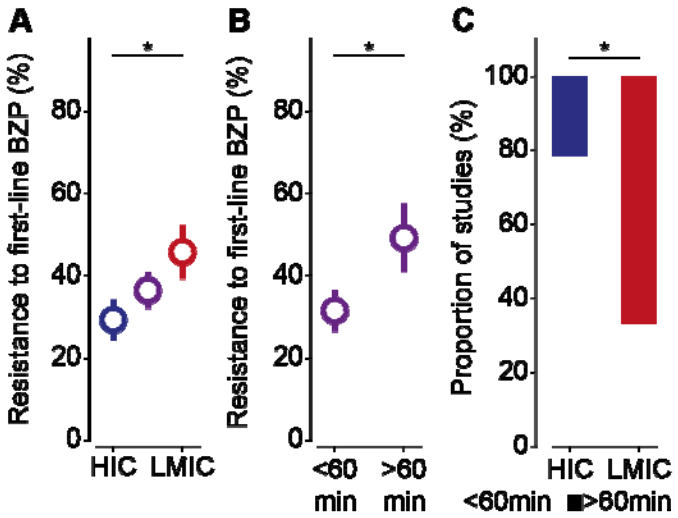
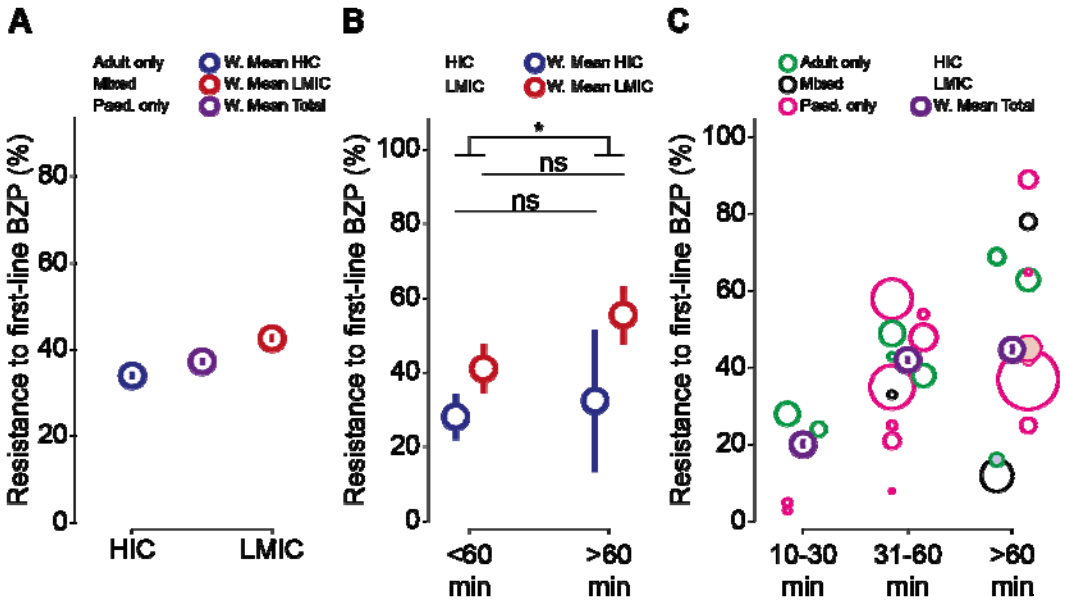


Fig.1: Socio-economic and temporal differences in benzodiazepine-resistant convulsive status epilepticus. A, Reported resistance to first-line benzodiazepines (BZPs) in convulsive status epilepticus (CSE) across countries with different economic profiles. 'Resource-equipped' countries are those classified as 'high-income countries' (HIC, blue) [68]. 'Resource-limited' countries included those classified as 'low-middle-income countries' (LMIC, red). Studies from LMIC reported higher resistance to first-line BZPs compared to studies conducted in HIC (LMIC: *mean* 45.71 ± *SEM* 5.97 % vs HIC: *mean* 29.22 ± *SEM* 4.33 %, p = 0.03, *unpaired t-test*). The mean reported resistance to BZPs across all studies (purple) is 36.44 %(± *SEM* 3.81 %). **B**, Studies in which the mean duration of CSE before first-line treatment was greater than 60 minutes reported higher resistance to first-line BZPs compared to studies in which the mean duration of SE was less than 60minutes (>60min: *mean* 49.18 ± *SEM* 7.71 min vs <60min: *mean* 31.47 ± *SEM* 4.51 min, p =0.03, *unpaired t-test*). **C**, Studies from LMIC are more like to report mean duration of CSE prior to first-line treatment > 60min compared to those from HIC (66.67% vs 21.43%, *OR* 7.33, p = 0.04, *Fisher-Exact test*). The original data and analysis code used to generate these figures are available in a freely accessible online repository. The supplementary figures accompanying this manuscript stratify studies according to the age group and number of study participants (weighted point estimates and error margins are included). The asterisks indicate significant differences at a $p < 0.05$ threshold.



Supp. Fig.1: Socio-economic and temporal differences in benzodiazepine-resistant convulsive status epilepticus stratified by age group and number of study participants. A, Reported resistance to first-line BZPs across countries with different economic profiles. 'Resource-equipped' countries are those classified as a 'high-income countries' (HIC, blue) while 'Resource-limited' countries are those classified as 'low-middle-income countries' (LMIC, red)[68]. Studies with adults only (green), children only (magenta) and those that combined both age groups ('Mixed', grey) are separated. The size of the symbol corresponds to the number of episodes of convulsive status epilepticus (CSE) reported in the study. The weighted means (W.Mean) and standard error of mean (*SEM*) for the HIC and LMIC (weighted by sample size and across both age groups) were calculated for each economic group (HIC: *mean* 33.95 ± *SEM* 0.31% vs LMIC: *mean* 42.55 ± SEM 0.5 %) as well as across all studies (*mean* 37.22 ± *SEM* 0.28 %. **B**, Resistance to first-line benzodiazepines (BZPs) reported at different phases of CSE. Studies were separated according to the estimated CSE latency being less than or greater than 60 minutes (<60 min vs >60min) and separated by economic groups. In HIC, resistance to first-line BZPs appears non-significantly higher in CSE >60 minutes (>60 min: *mean* 32.33 ± *SEM* 18.37 % vs <60min: *mean* 28.0 ± *SEM* 5.44 %, p = 0.18, *unpaired t-test*). In LMIC, resistance to first-line BZPs also appears non-significantly higher in CSE >60 minutes (>60 min: *mean* 55.5 ± *SEM* 7.72 % vs <60min: *mean* 41.0 ± *SEM* 6.56 %, p = 0.26, *unpaired t-test*). When comparing across economic groups, however, reported resistance to BZPs is significantly higher is CSE is > 60 minutes (data shown in Fig.1, p = 0.03). **C**, Further separation of difference phases of CSE based on estimated duration prior to first-line treatment (10 – 30 min: weighted *mean* 20.0 ± *SEM* 0.61 %; 31 – 60 min: *mean* 41.97 ± *SEM* 0.36 %; >60 min: *mean* 44.71 ± *SEM* 0.66 %).

Dosing for first-line BZP trials were not necessarily consistent across studies. Theoretically, to be deemed BZP-resistant, a patient in SE should show no response to a BZP even if given the maximum safe total dose. In reality, however, many patients often do not receive adequate doses of BZPs with this being particularly pertinent for out of hospital SE (Guterman et al 2020)[69]. This under-dosing may be attributed to the administration route, since, for example, rectal administration is less well absorbed than intravenous administration (Alldredge et al, 2001; Silbergleit et al, 2012), or clinicians choosing to administer an alternative ASM instead of a second dose of BZP. Moreover, some care providers are overly cautious in administering BZPs out of concern about causing respiratory depression that would necessitate ventilatory support even though there is currently no evidence to support this in the context of CSE (Guterman et al, 2020).

Duration of SE appears to be an important determinant of response to BZPs[14, 70]. This indicates that the pathophysiology of SE may involve adaptive changes in the brain that occur across different stages in the evolution of SE, ultimately affecting the efficacy of BZPs. Understanding the sequence in which such changes occur may provide important insights into how the treatment of SE can be optimised. BZPs target the $GABA_A$ receptor ($GABA_AR$). Consideration, therefore, of the structure and function of this chloride (Cl^-) permeable inotropic receptor is important for understanding how BZP resistance might emerge in SE.

The $GABA_AR$ receptor: a Cl^- channel sensitive to benzodiazepines

The $GABA_AR$ is a pentameric ligand-activated, ionotropic receptor that is formed by different permutations of its five constitutive subunits[71, 72]. It is largely, but not exclusively, expressed on the postsynaptic membrane of neurons. The different subunits are separated into classes according to their varied amino acid composition (α, β, γ, δ, ε, π, θ). Furthermore, some of these can be sub-classified into different isoforms (α_{1-6}, β_{1-3}, γ_{1-3}). The multiplicity of subunit classes and isoforms ultimately determines the biophysical properties of the channel including its localisation, ligand binding and conductance. The most common arrangement found in the brain is two α_1, two β_2 and a γ_2 subunit[73]. These receptors are associated with phasic inhibition and can be located at most GABAergic synapses (**Fig.2**)[88].

The γ subunit is considered crucial for $GABA_AR$ clustering at synapses[75]. Consistent with this, $GABA_ARs$ where the γ subunit has been replaced by a δ subunit are found at extrasynaptic sites[76]. $GABA_ARs$ are activated by the neurotransmitter GABA, which binds between the α_1 and β_2 subunits. This induces a conformational change in the pentameric channel to make it selectively permeable to Cl^- and, to a much lesser extent, to bicarbonate (HCO_3^-)[77–79]. Cl^- flux predominates and, under physiological conditions, the transmembrane electrochemical gradient favours Cl^- movement into the cell. $GABA_AR$ activation therefore typically causes a net inward movement of negative charge and membrane hyperpolarisation (**Fig.2A**). This underlies the 'classic' inhibitory action of $GABA_ARs$.

The function of the $GABA_AR$ can be enhanced or attenuated using various pharmacological manipulations[71 ,72, 80]. BZPs, formed from the union of the benzene and diazepine chemical rings[81], are a class of synthetic $GABA_AR$ positive allosteric modulators that can enhance $GABA_AR$ conductance. By enhancing GABAergic signalling, BZPs typically have anti-seizure, sedative, hypnotic and anxiolytic properties. The effect of BZPs is determined by the different subunit configurations of the $GABA_AR$ and their relative distribution throughout the CNS. Furthermore, there are distinct pharmacological profiles for the different BZP agents, which relate to their different binding affinities to various $GABA_AR$ isoform configurations. There is also an endogenous equivalent to BZPs known as the endozepines[82, 83]. These compounds are released by astrocytes and are able to positively modulate GABAergic signalling[84].

BZPs bind to the $GABA_AR$ between its α and γ subunits[85]. Effective binding depends upon a key histidine residue within the α subunit[86]. This structural requirement for BZP binding is present in all isoforms of the α subunit except α_4 and α_6[87]. Newer BZP agents are able to target specific α subunits[88]. Upon binding, BZP increases the affinity of the receptor to GABA[89–91]. This results in an increase in the frequency of channel opening, thereby increasing the conductance of the $GABA_AR$[92]. Under typical conditions, this facilitates the influx of negatively charged Cl^- ions, enhancing the inhibitory actions of GABA by making it less likely that neurons fire action potentials (**Fig.2B**). This is the putative mechanism by which benzodiazepines are thought to stop seizures. The ultimate effect of benzodiazepines, however, is dependent on the functional properties of $GABA_ARs$, which can shift with progressive seizure activity.

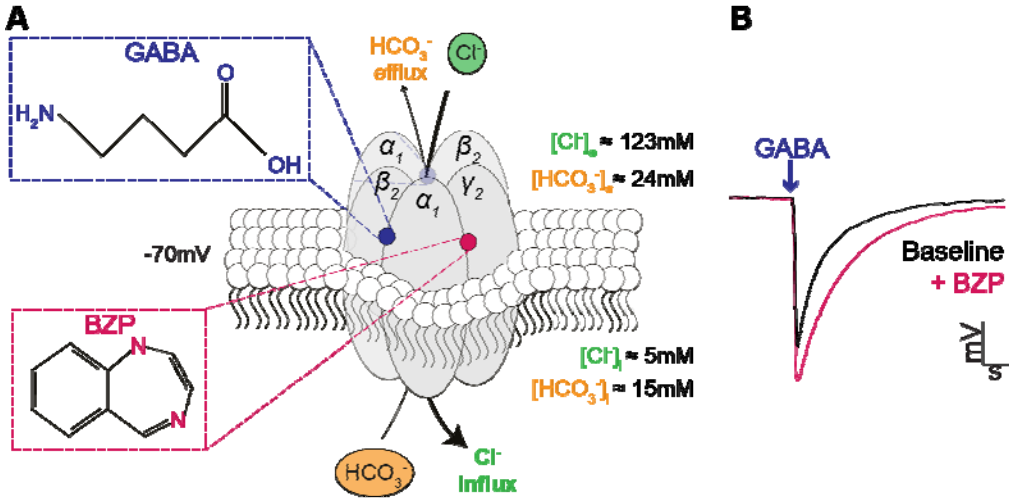


Fig.2: **BZPs bind to Cl^- permeable $GABA_AR$s and enhance channel conductance. A**, The GABA-A receptor ($GABA_AR$) is a pentameric channel that is found on the neuronal membrane. It is formed from different combinations of subunits with the most common configuration being α_1- β_2- γ_2- α_1- β_2. The channel is activated by the binding of the neurotransmitter, γ-amino butyric acid (GABA, blue), at the junction between α1 and γ2 subunits. The open channel allows for Cl^- (green) and HCO_3^- (orange) flux along their respective electrochemical gradients and is four times more permeable to Cl^- ions compared to HCO_3^- ions. Under typical conditions (i.e. when the resting membrane potential is approximately -70mV), $GABA_AR$ permit Cl^- influx (thick black arrow) and HCO_3^- efflux (thin black arrow). The benzodiazepines (BZP, magenta) are a class of $GABA_AR$ allosteric modulators that bind onto the $GABA_AR$ at the junction of its subunits. Under baseline conditions there is typically a low intracellular concentration of Cl^- ($[Cl^-]_i$). **B**, When $GABA_AR$s are activated, the predominant flux of ions is Cl^- movement down its electrochemical gradient into the cell. This influx of negative charge causes a membrane hyperpolarisation referred to as an inhibitory postsynaptic potential (IPSP). BZPs broadly increase the conductance of the $GABA_AR$s by increasing the frequency at which the $GABA_AR$ opens. This increases the amplitude and duration of the IPSP (magenta).

$GABA_AR$ function is dependent on the state of the transmembrane Cl^- gradient

The $GABA_AR$ is primarily a Cl^- channel. Therefore, the effects of positively modulating its conductance via BZP binding are governed by the state of the transmembrane Cl^- concentration gradient. Evidence from *in vitro* and *in vivo* studies using both animal and human tissue has shown that this gradient is dynamic and can change considerably as a function of development and state of network activity[93–98].

The resting transmembrane Cl^- gradient and consequently the Cl^- equilibrium potential is established by multiple cellular factors including the Na^+/K^+ ATPase, impermeant anions, Cl^- conductances and Cl^--cation cotransporters (CCCs)[93–95, 99–103]. However, only secondary active transport mechanisms for Cl-, such as the Cl^--cation cotransporters, are able to establish a driving force for Cl^-. That is, they are able to shift the Cl^- equilibrium potential away from the resting membrane potential, thereby controlling the properties of $GABA_AR$ mediated signalling (Düserwald et al, 2018). The Na^+-K^+-Cl^- co-transporter (NKCC1) typically results in Cl^- influx and a more positive Cl^- equilibrium potential relative to the resting membrane potential, whereas the K^+-Cl^--co-transporter 2 (KCC2) extrudes Cl^- resulting in a more negative Cl^- equilibrium potential relative to the resting membrane potential. During development, there is differential expression and change in the function of these CCCs. In the immature brain, KCC2 expression within neurons is low compared to NKCC1 expression (Blaesse et al, 2009). This results in a higher intracellular concentration of Cl^- in younger neurons, compared to older neurons, which causes GABAergic signalling to be depolarising. As neural tissues mature, there is a relative up-regulation in KCC2 within neurons (Rivera et al, 1999). In this mature state, there is increased Cl^- extrusion, which results in a lower intracellular Cl^- concentration ($[Cl^-]_i$) and an inhibitory shift in GABA function. In rodents this transition from GABAergic depolarisation to hyperpolarsiation has been shown to occur at

approximately the end of the first postnatal week. As the human nervous system is relatively more mature at birth compared to rodents, it is thought that GABA is already hyperpolarizing in healthy human cortex at term (Löscher and Kaila, 2021). Understanding the role of neuronal NKCC1 during development and disease is complicated because, unlike KCC2, transcriptomic signals for NKCC1 (and therefore likely expression) are higher in oligodendrocytes and endothelial cells than in neurons in both mouse and human brain tissue (Virtanen et al, 2020; , Saunders et al, 2018; Bakken et al, 2020). That said, NKCC1, does impact Cl^- levels and GABAergic responses in neurons. For example, NKCC1 appears to contribute to specific sub-cellular compartmental effects such as raised Cl^- in axons (Khirug et al, 2008) that result in depolarising GABAergic responses to inhibitory neurons which target the axon initial segment (Szabadics et al, 2006).

Seizures can change the expression and activity of both KCC2 and NKCC1, with these effects developing over tens of minutes to hours. Multiple *in vitro* and *in vivo* studies have shown that ongoing seizure activity induces a decrease in the function and surface expression of KCC2, which reduces the Cl^- extrusion capacity of neurons (Lee et al, 2010; Lee et al, 2011)[36, 104–106]. This is accompanied by an increase in the relative expression and activity of NKCC1, which acts as an inward transporter of Cl^-[107–109]. For example, Barmashenko et al (2011) have shown bumetanide sensitive (bumetanide is a NKCC1 specific antagonist at low concentrations) depolarising GABA responses in neurons following SE in rats as well as corresponding shifts in the ratio of KCC2 to NKCC1 mRNA expression. These seizure-associated changes in CCCs have also been observed *in vitro* in human brain tissue from patients with intractable epilepsy caused by different aetiologies[110–114].

Therefore, prolonged seizure activity, of at least tens of minutes, appears to induce a reversal of the relative expression of Cl^- cotransporters, similar to expression patterns observed earlier in development. These changes increase baseline Cl^- levels, but also render neurons more susceptible to activity-induced Cl^- accumulation. Taken together, such alterations are predicted to weaken $GABA_AR$-mediated inhibition and thus reduce the potential to enhance inhibition through allosteric modulation of the receptor by BZPs.

While CCCs primarily determine the baseline $[Cl^-]_i$, they also influence whether Cl^- accumulates in neurons over shorter time scales (seconds to minutes), which is associated with increased network activity. During relatively quiescent periods, $[Cl^-]_i$ is low (typically around 5mM), which equates to a reversal potential for the $GABA_AR$ (termed E_{GABA}) of approximately -70mV. When $GABA_AR$ is activated, the transmembrane Cl^- gradient favours Cl^- influx, causing membrane hyperpolarisation and an inhibitory action via the $GABA_AR$. KCC2 uses the transmembrane K^+ gradient to extrude Cl^- in order to maintain low $[Cl^-]_i$ and hence maintain E_{GABA} at levels negative to the resting membrane potential. Under these conditions, the inhibitory function of the $GABA_AR$ is preserved[115].

Investigations in animal models have shown that increasing levels of network activity, whether physiological or during the build-up to seizures, causes enhanced synaptic GABA release and $GABA_AR$ activation[116, 117]. This strong $GABA_AR$ activity generates large Cl^- influxes that cause rises in $[Cl^-]$ [118-122] . Such Cl^- influx is enhanced when $GABA_AR$ activation is combined with concomitant membrane depolarisation via glutamate receptors[123]. E_{GABA} therefore can become more positive relative to the resting membrane potential (V_m), although remaining below the action potential threshold. In this state, $GABA_AR$-mediated signalling is weakened and will either inhibit (by “shunting”) or facilitate the effect of simultaneous glutamate receptor activation depending on the relative location and timing of synaptic inputs[124, 125]. These conditions are accompanied by increased K^+ extrusion and a rise in the $[K^+]_e$[126]. If network activity increases further as is seen during seizures, the combined effect of increasing $[Cl^-]_i$ and $[K^+]_e$ can overwhelm the Cl^- extrusion capabilities of KCC2[127]. This increased Cl^- accumulation depolarises E_{GABA} beyond the action potential threshold[36, 128]. In this state, subsequent $GABA_AR$ activation can be sufficiently depolarising

that it will trigger action potentials[36, 128]. In other words, GABAergic signalling will have become excitatory (**Fig.3**).

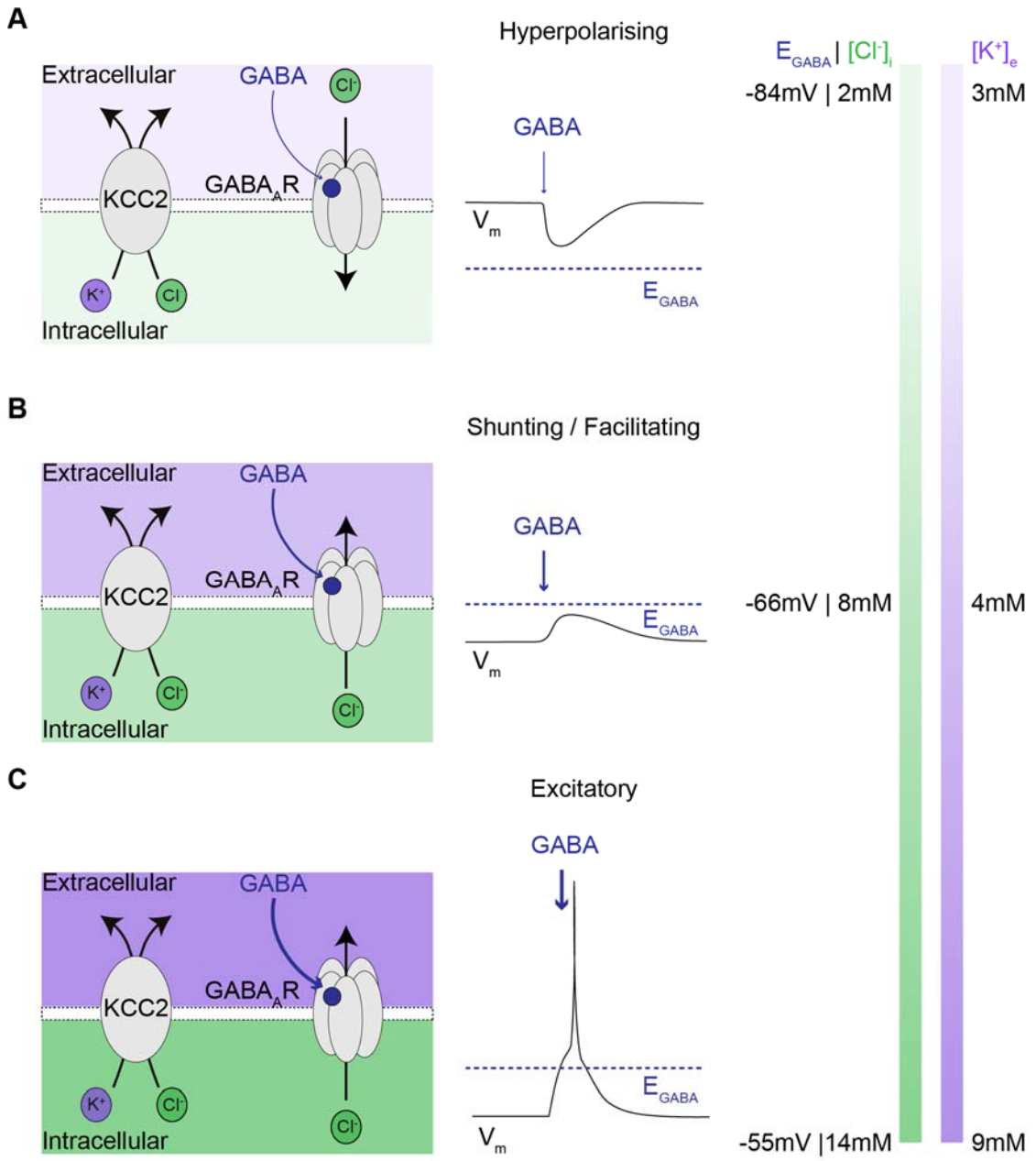


Fig.3: Changes in intracellular Cl^- concentration set the properties of $GABA_AR$-mediated signalling. A, At rest, there is a low concentration of intraneuronal chloride ($[Cl^-]_i$) and reversal potential for GABA (E_{GABA}) is hyperpolarised relative to the resting membrane potential (V_m). This allows Cl^- influx and membrane hyperpolarisation. The low concentration of extraneuronal potassium ($[K^+]_e$) allows for efficient KCC2-mediated extrusion of Cl^-. **B**, During states of increased network activity, more GABA is released into the synaptic cleft leading to enhanced $GABA_AR$ activation, greater Cl^- influx and a rise in the $[Cl^-]_i$, shifting the E_{GABA} to sit above V_m but below the AP threshold. Subsequent $GABA_AR$ activation can then result in net anion efflux and GABA mediating shunting or facilitation of accompanying glutamatergic synaptic input. There is also increased KCC2 activity that acts to correct for the raised $[Cl^-]_i$, which may lead to increased $[K^+]_e$. $[K^+]_e$ is also increased by K^+ efflux via other channels during network activity. **C**, When hyperexcitability is sustained, Cl^- accumulation can be so severe that E_{GABA} shifts above the AP threshold and $GABA_AR$ activation becomes excitatory and can trigger APs. The rising $[K^+]_e$ reduces the transmembrane K^+ gradient further, which impedes KCC2 function and facilitates the rise in $[Cl^-]_i$.

What causes BZP resistance?

The pathophysiology of BZP resistance during SE can broadly be classified into either inherited or acquired causes. The inherited causes relate to mutations in the genes that encode for $GABA_AR$s (**Box 1**). The acquired causes can be further sub-classified. The first category relates to pharmacokinetic and pharmacodynamic tolerance to BZPs that occur independent of SE (**Box 2**). The second, relate to the changes to $GABA_AR$ physiology because of widespread network hyperexcitability. Whilst it is likely that these different aspects are operating in concert, our review will focus on the activity-dependent changes to the GABAAR that occur throughout the evolution of SE.

Box 1: Genetic mutations in $GABA_AR$s affect BZP sensitivity

There are various mutations of the $GABA_AR$ that directly affect BZP binding and could therefore contribute to BZP resistance in SE. A mutation in the γ_2 subunit, [γ_2(R43Q)], increases the rate of desensitisation of the receptor to BZPs[129]. Various mutations in $GABA_AR$ subunit configuration can cause disruptions in the interface between the γ and β subunits, which negatively affect channel function[89]. In addition, some mutations may cause an increase in γ subunit trafficking, thereby decreasing the availability or function of BZP-sensitive $GABA_AR$s at the synapse[130]. These mutations, however, are typically associated with epileptic encephalopathies, such as Dravet syndrome[131, 132], and therefore would likely relate to a select number of patients in SE within the context of distinct electroclinical syndromes.

Box 2: BZP-related pharmacokinetic and pharmacodynamic tolerance

Acute or previous chronic exposure to BZPs or other compounds (including ASMs) can reduce the efficacy of BZPs, with individualised susceptibility to this phenomenon[133, 134]. Evidence from both experimental and clinical studies demonstrate how this can initially occur by induction of pharmacokinetic tolerance[135]. Pharmacokinetic tolerance refers to any mechanism by which medications could change the bioavailability of the BZPs. For instance, many ASMs share common breakdown pathways via the cytochrome P450 enzyme system[136, 137]. People with epilepsy who have received treatment with, for example, carbamazepine, phenytoin, and phenobarbital (all known to induce the cytochrome P450), would likely need higher doses of BZPs as first-line agents to treat SE, owing to the induced increase in the ability to break down BZP[138]. Another important consideration is the baseline physiology of the patient and any other co-morbid disease (especially those affecting hepatic and renal function) that would further impact the metabolism of BZPs (Griffin et al, 2013). In contrast, pharmacodynamic tolerance refers to how the sensitivity of the $GABA_AR$ to BZP changes after acute or chronic exposure[139]. Evidence from studies in both animals and humans has shown that both short- and long-term BZP use causes changes within the central nervous system that ultimately affect the ability of the $GABA_AR$'s to be positively modulated by these agents[139, 140] (Rosenberg and Chiu, 1985; mice by Wong et al, 1985). Multiple studies have demonstrated how tolerance to the sedative, hypnotic and anti-seizure effects of BZPs can emerge relatively rapidly, while the anxiolytic effects appear to be more resistant[141–143]. Evidence from animal and human studies suggest that continued BZP use could drive multiple downstream effects that culminate in BZP tolerance. First, persistent exposure to BZPs leads to a loss of allosteric coupling between GABA and BZP binding sites on the $GABA_AR$, potentially via changes in receptor assembly or phosphorylation patterns[144]. Second, there may be alterations in the assembly, membrane trafficking and synaptic accumulation of $GABA_AR$s[145]. Third, there may be compensatory changes in glutamatergic neurotransmission[146]. Fourth, there may be interactions between various G-coupled protein receptors and the $GABA_AR$ through concurrent activation of serotonergic[147], dopaminergic[148] and muscarinic[149] pathways. Lastly, BZPs have also been shown to cause changes in neurosteroid signaling[150].

SE initiates a cascade of activity-dependent changes that reduce the efficacy of BZPs

In addition to pre-existing inherited and acquired factors, evidence from animal models indicate that $GABA_AR$s undergo significant changes during SE including alterations in receptor number at inhibitory synapses and receptor subunit composition, which can affect BZP binding (**Fig.4A**). SE also causes dynamic shifts in ion gradients that can result in the $GABA_AR$ shifting from mediating inhibition to exacerbating continued excitation (**Fig.4B**). These structural and functional alterations to the integrity of the $GABA_AR$ occur in parallel throughout the evolution of SE and cause progressive resistance to BZPs (**Fig.5**).

During SE there appears to be internalisation and reconfiguration of the $GABA_AR$ that leads to a marked reduction in BZP sensitivity starting several minutes after the onset of seizure activity. After 10 minutes of SE in *in vivo* animal models, there is increased mobility and internalisation of the synaptic, BZP-sensitive configuration of the $GABA_AR$[151-154]. This phenomenon has been demonstrated with different techniques in multiple systems utilising both optical and electrophysiological measures of $GABA_AR$ function. More specifically, seizure activity has been shown to cause a down-regulation of the $\alpha_{1\text{-}4}$, $\beta_{2\text{-}3}$ and γ_2 subunits, which are essential to form the BZP binding site[153-155]. Concurrently, the expression of extra-synaptic, BZP-insensitive $GABA_AR$s increases, as demonstrated by an upregulation of α_5 and δ subunits that are typically responsible for tonic $GABA_AR$ inhibition[151,154,156]. Collectively, these represent an acquired change in $GABA_AR$ structure that contributes to BZP resistance occurring over the course of minutes to hours of ongoing seizure activity.

Animal studies have shown that receptor internalisation can start to develop after 10 minutes of SE[157], and becomes more pronounced at 30 minutes[151] and 60 minutes[152]. These seizure-induced changes in the benzodiazepine sensitivity of $GABA_AR$ can be long lasting. For example, resected brain tissue from patients with multi-drug resistant temporal lobe epilepsy and who have experienced recurrent seizures for many years show decreased expression of $GABA_AR$s with BZP binding sites as compared to tissue from autopsies from neurologically normal patients or healthy matched controls[158, 159]. Positron emission tomography (PET) studies have allowed analysis of human $GABA_AR$ composition *in vivo* demonstrating that in people with refractory epilepsy changes in BZP-binding affinity are a key feature of the site of seizure origin, the so-called 'ictogenic focus'[160,161].

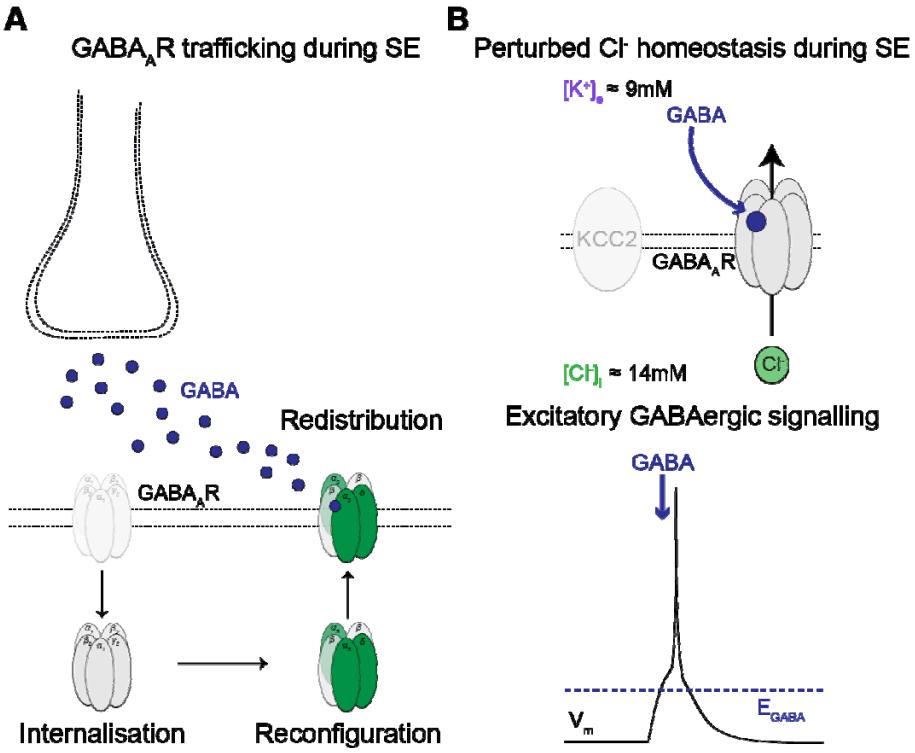


Fig.4: SE causes disruptions to GABA$_A$R composition and function that may underlie acquired resistance to BZPs. A, During status epilepticus (SE), the GABA-A receptor (GABA$_A$R) undergoes an endocytosis-mediated internalisation. Once internalised, GABA$_A$Rs are reconfigured with subunits insensitive to BZPs, which are preferentially redistributed to extrasynaptic locations. **B**, SE leads to an activity-dependent increase in the concentration of intraneuronal chloride ([Cl$^-$]$_i$) and extraneuronal potassium ([K$^+$]$_e$), as well as an acquired dysfunction of the potassium-chloride-co-transporter (KCC2). Together, these lead to a depolarising shift in the Cl$^-$ electrochemical gradient that can cause GABA$_A$R activation to become excitatory.

Given the efficacy of GABA$_A$R inhibition relies upon the transmembrane gradient for Cl$^-$, activity- (i.e. seizure-) dependent changes in the intraneuronal concentration of chloride can subvert GABA-$_A$R inhibitory signalling and sustain abnormal network activity. This short-term change in GABA$_A$R signalling as a function of a change in the transmembrane Cl$^-$ gradient has been referred to as 'short-term ionic plasticity'[123, 126]. Such short-term, activity-dependent excitatory shifts in GABAergic signalling can occur during both self-terminating and self-perpetuating seizure activity[36, 127, 162–164]. This process is further aggravated by seizure-induced changes in the expression of CCCs, which make neurons more susceptible to Cl$^-$ accumulation.

Given the conditions of profound Cl$^-$ loading that have been shown to occur during seizures and SE using animal models, it is predicted that BZPs could lose their efficacy or even perhaps exacerbate seizure-like activity by enhancing excitatory GABAergic signalling[36,128,163,165]., Deeb et al[165] have demonstrated, in dissociated neuronal cultures, that Cl$^-$ accumulation during ongoing network activity reduces the inhibitory effect of diazepam. More recently, *in vitro* brain slice models have shown that SE-induced increases in Cl$^-$ and the resulting excitatory shift in GABAergic signalling associates with a progressive loss in the efficacy of diazepam[36]. In addition, in brain slices with progressive SE-like activity and Cl$^-$ loaded neurons, the application of diazepam exacerbated the severity of epileptiform discharges.

Computational modelling of ion dynamics during seizures has implicated increasing intraneuronal Cl$^-$ in extending seizure activity and contributing to the development of SE[166]. The important role of Cl$^-$ in the pathogenesis of SE and efforts to manipulate Cl$^-$ extrusion are, therefore, of increasing therapeutic interest[167,168]. For example, recent studies have

explored manipulating Cl influx and efflux to study how this affects the evolution of seizure activity. Attempts have been made to modulate KCC2 function through overexpression[169] or by preventing seizure-induced phosphorylation-dependent KCC2 inactivation[170] with both approaches significantly limiting the severity of seizure activity. In addition, pharmacologically blocking NKCC1 in animal models can rescue BZP sensitivity in SE[171].

Taken together these experimental and computational data suggest that during SE there is a preferential shift away from phasic, BZP-sensitive GABAergic inhibition towards tonic, BZP-insensitive GABAergic excitation. There are multiple processes involved and likely occurring in parallel across different timescales from minutes to hours (see **Fig.5**). These insights, gleaned from basic epilepsy research, likely explain the clinical phenomenon of progressive BZP-resistance emerging in SE of prolonged duration (as shown in **Fig.1B**). Changes to the $GABA_AR$ configuration and the capability of neurons to extrude Cl^- are also likely to persist after the termination of SE potentially contributing to the development of epilepsy and persistent BZP-insensitivity in these individuals.

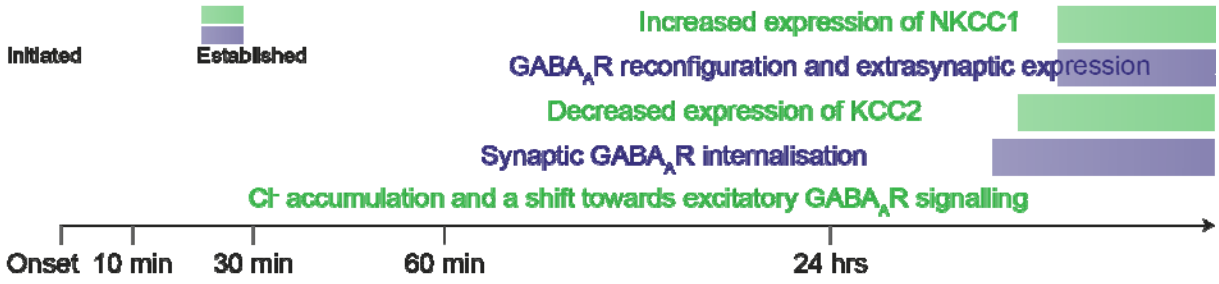


Fig.5: Proposed timeline of changes affecting BZP efficacy during SE. BZP, benzodiazepine; $GABA_AR$, $GABA_A$ receptor; KCC2, K+-Cl—co-transporter; NKCC1, Na+-K+-Cl—co-transporter. Green refers to alterations to Cl- homeostasis while the blue highlights changes to $GABA_AR$ expression.

The changes in the function of $GABA_AR$-mediated inhibition are also linked to glutamatergic signalling through the N-Methyl-D-aspartate receptor (NMDA-R). During SE, widespread and persistent activation of NMDA-Rs causes an increase in intracellular calcium ($[Ca^{2+}]_i$) (DeLorenzo et al, 1998). This rise in $[Ca^{2+}]_i$ then activates multiple second-messenger pathways (e.g. protein kinase C, calcineurin and extracellular signal-regulated kinases), which can decrease the expression of both phasic and tonic $GABA_ARs$ through complementary pathways (Bannai et al, 2015; Terunuma et al, 2008; Joshi and Kapur, 2013; Eckel et al, 2015; Bannai et al, 2015). In addition, NMDA-R-mediated Ca^{2+} influx can impede KCC2 function thereby reducing the inhibitory capacity of the $GABA_ARs$ that are expressed (Lee et al, 2011). The elevated $[Ca^{2+}]_i$ activates mechanisms that upregulate the expression of more NMDA-Rs as well the other main glutamatergic receptor, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA-R) (Naylor et al, 2005; Rajasekaran et al, 2012). The net result is an enhancement of glutamatergic excitation combined with a reduction in GABAA-R mediated inhibition (Burman et al, 2020).

A new, more nuanced approach may be needed to treat SE

Experimental data suggest that resistance to BZPs involves multiple mechanisms that affect $GABA_AR$ function and operate on a range of timescales, including the timescale of an individual SE episode. This is supported by the clinical observation that episodes of SE longer than 60 minutes appear to show greater resistance to BZPs (**Fig.1**). Therefore, an argument could be made that patients who present in SE that has lasted over 60 minutes, or patients that have previously presented in SE, may benefit from a more tailored treatment approach that may not include BZPs as first-line management. Such a strategy has the potential to speed the delivery of the most efficacious interventions and thus help reduce the morbidity and mortality associated with prolonged SE. Whilst an appealing concept in theory,

practically there is as of yet no clinical evidence to support the use of alternative ASMs as first-line management for SE. BZPs remain the gold standard as they are cheap, safe and effective if given at the correct time and in adequate dose.

If one were to, however, consider a possible candidate as an alternative ASM as first-line treatment, what should this be? One rational approach to this question would be to consider the most effective second-line agent in the case of BZP-resistance in SE. Recently, there have been large multi-centre studies exploring the efficacy of second-line agents in both adult and paediatric patients with convulsive SE[9-11]. The recent Established Status Epilepticus Treatment Trial (ESETT) showed that in people who received second-line treatment with either levetiracetam, valproate or fosphenytoin, over 50% did not respond to treatment[9, 31]. One agent, however, that has been excluded from such studies as a second-line treatment option is phenobarbital (PHB). Whilst at low doses this agent is also a $GABA_AR$ agonist, at high doses it is very effective at terminating persistent SE-like activity in animal models[36]. This is attributed to pharmacological effects in addition to its action on $GABA_ARs$. At higher concentrations, PHB is also an effective antagonist of AMPA and kainate glutamatergic receptors[173–175]. This means that PHB may maintain anti-seizure activity, even in brain areas of modified $GABA_AR$ expression or with profound intraneuronal Cl^- accumulation.

PHB has been shown to be an effective agent for the treatment of refractory CSE and is still widely used in resource-limited healthcare systems. For example, in an adult cohort of patients intravenous phenobarbital was effective in 81% of those presenting in BZP-resistant CSE and more effective than intravenous valproate that was only effective in 44% of cases[176]. In children with BZP-resistant CSE, PHB has been reported to be effective in 86% of SE and more effective than the more widely adopted phenytoin[8]. While there is a concern that respiratory depression can follow a bolus injection with PHB, this appears to occur in only a small number of patients (~13% in adults {Treiman et al 1998} and ~7% in paediatric patients {Burman et al 2019}) which is not significantly different from other ASMs used in the management of SE.

Chronic PHB can associate with neurobehavioural and cognitive adverse effects[177–183]. There is, however, no data to support that the same occurs when PHB is used in the acute setting[184–188]. This should inform cost-benefit calculations given the need to stop SE against potential negative effects upon cognition. This consideration is already well established in other clinical situations such as the acute use of valproate to manage SE in pregnant women despite its' well-known teratogenicity[189]. A major barrier to the further use of PHB, especially in resource-limited countries, is that suppliers have reduced production due to the limited profitability as well as the restrictive regulations for access to barbiturates[190].

Lastly, one may also consider moving away from first-line monotherapy with BZPs and instead combine BZPs with other agents that exhibit synergistic effects. There are new, emerging treatment options that may prove to be more effective in safely terminating SE, which target more specific mechanisms related to SE pathophysiology (Trinka et al, 2016; Amengual-Gual et al, 2019; Neligan et al, 2021). Specifically, clinically available agents that target NMDA-Rs (i.e. ketamine) and AMPA-Rs (i.e. perampanel) are appealing prospects as these receptors appear to be upregulated in SE whilst also contributing to the degradation of $GABA_AR$-mediated inhibition (Kapur et al, 2018; Leo et al, 2018; Prisco et al, 2020). To date, however, there is insufficient evidence to support the use of these agents in the early management of SE, but this may change with the completion of ongoing clinical trials (Brigo et al, 2018; Rosati et al, 2018; Vossler et al, 2020). There has also been encouraging evidence from animal studies that have shown a revival of BZP efficacy in models of resistant SE, when the BZPs are combined with agents that target other systems. These include combining a BZP (either diazepam or lorazepam) with the NMDA-R competitive antagonist 3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP)[88] or the K^+ channel

activator flupirtine[192]. Further clinical studies into the use of these synergistic treatment combinations are needed.

Prevailing mismatches between the bench and the bedside: unanswered questions

In this review, we have presented both clinical and experimental data that highlight the significance of BZP-resistant CSE and the mechanisms that likely underlie this clinical phenomenon in adults and children. The relevance of these insights into other forms of SE, namely nonconvulsive SE (NCSE) and neonatal SE are briefly discussed in **Box 3** and **Box 4**.

Box 3: Non-convulsive Status Epilepticus

NCSE occurs when there is continuous or repetitive seizure activity seen electrographically with or without cognitive and behavioural changes, but without any motor (convulsive) manifestations[4, 193]. CSE and NCSE can exist in a continuum whereby a patient can transition from CSE to non-convulsive SE (NCSE), and vice versa. Unlike the vast amount of literature on the use of BZPs in the management of CSE, there is a dearth of studies on the management of NCSE. This is likely due to the difficulties in diagnosing this condition outside of a setting where there is access to continuous EEG monitoring[194]. Overall, 67% of patients in NCSE do not respond to first-line treatment with BZPs[64, 195–197]. This rate is approximately 1.5 times higher compared to CSE and likely, at least in part, due to delays in recognition and treatment initiation for NCSE.

Box 4: Neonatal Status Epilepticus

Neonatal SE is best considered as a separate entity to paediatric and adult SE, as synaptic signalling mechanisms are considerably different in neonates compared to those in the paediatric and adult brain[93, 95,198,199]. There is differential expression of the Cl^- cotransporters throughout development resulting in potentially higher levels of Cl^- in the neonatal brain, causing less inhibitory, or depolarising, GABAergic signalling. This is combined with a relatively smaller contribution of glutamatergic synaptic activity under physiological conditions[200–203]. With ongoing development, KCC2 is upregulated, lowering Cl^- and promoting inhibitory GABAergic signalling that balances associated maturation in the number and strength of glutamatergic synapses[204–207]. The result of higher intraneuronal Cl^- in the neonatal brain is that positive allosteric modulators of $GABA_AR$s are less effective in terminating seizure activity, and could possibly exacerbate SE[208]. For example, a common feature of neonatal SE is that there is often no clinical presentation accompanying the electrographic seizure activity, particularly in very sick or preterm neonates[209]. This phenomenon, often referred to as 'electroclinical uncoupling', can also be induced by the administration of $GABA_AR$ modulators, like BZP or low-dose PHB[210–216]. This may be attributed to regional differences in intraneuronal Cl^- concentration. For example, Glykys et al[217] have shown that a lower intraneuronal Cl^- favouring $GABA_AR$-mediated hyperpolarisation, first emerges in subcortical regions before cortical regions. This more nuanced understanding of GABAergic signalling in the neonatal brain, particularly regarding the potential role of neuronal NKCC1 has inspired further exploration into how manipulating this cotransporter might affect neonatal seizures and potentially rescue anti-seizure effects of $GABA_AR$ modulators[208, 218–222]. Clinical trials have investigated whether blocking NKCC1 with bumetanide has a measurable clinical benefit on neonatal seizures[223, 224]. Due to mixed outcomes and safety concerns, further data is still needed to confirm whether the use of adjuvant bumetanide is safe and effective in the management of neonatal SE (Stafstrom et al, 2020).

Trying to bridge the gap between clinical and experimental domains of SE is a challenge and there remain unanswered questions around how BZP responsiveness can vary across different types and durations of SE. For example, whilst there are experimental and clinical

data that provide an explanation of how BZP resistance increases with duration of SE, there are also many cases where people present in prolonged CSE and yet still respond to first-line BZPs. Similarly, in individuals who appear resistant to first-line treatment with BZPs, many will still be sensitive to an infusion of midazolam (MDZ)[13]. This remains poorly understood. Based on experimental studies, one possible explanation for this varied BZP sensitivity in patients could be that across the brain there are differential responses to these agents, with some areas being BZP resistant and other areas remaining BZP sensitive (**Fig. 6**). For example, in areas of actively seizing neuronal networks with raised intraneuronal $[Cl^-]_i$ and extraneuronal $[K^+]_e$, GABAergic signalling would be excitatory and BZP ineffective. In contrast, in other less affected areas $[Cl^-]_i$ might be low and GABAergic inhibition would be intact. BZP would enhance inhibition in these brain areas. The combined effect of BZP would therefore be a function of which, and to what extent, different brain areas have been recruited into the seizure. These ideas are supported by recent computational modelling studies of seizure propagation dynamics that demonstrate how area-specific inhibitory capacity direct the temporal and spatial spread of activity[225, 226].

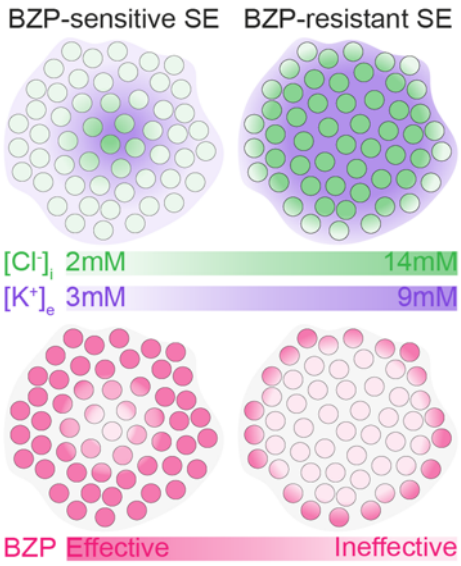


Fig.6: Spatial dynamics of activity-dependent shifts in $[Cl^-]_i$ and $[K^+]_e$ may explain different responses to BZPs. Cartoons show different epileptic networks, one where the network is sensitive to benzodiazepines (BZPs, left) and one where the network is resistant to BZPs (right). If the seizure focus is surrounded by areas with intact chloride (Cl^-) and potassium (K^+) transmembrane gradients (left), BZPs may be effective in preventing seizure propagation and thereby facilitate termination. If however, these gradients are compromised in a large enough area (right), BZPs would be ineffective in stopping seizure activity and could even help maintain seizure activity via excitatory GABAergic signalling. $[Cl^-]_i$, intraneuronal chloride; $[K^+]_e$, extraneuronal potassium.

It needs to be reiterated that the translation from 'bench to bedside' is rarely seamless. This is evident in numerous potential novel treatments which may work in animal models of SE but fail to generate any meaningful clinical benefit. One notable example of this is in the use of bumetanide to treat neonatal SE (see Box 4). Another is the recent investigation into the use of neurosteroids to treat SE (Rogawski et al, 2013). The shift from synaptic, phasic $GABA_AR$ to extrasynaptic, tonic $GABA_AR$ during SE (**Fig.4A**) offers an additional therapeutic target. Neurosteroids, such as allopregnanolone, which selectively target extrasynaptic $GABA_AR$ (Stell et al, 2001), have demonstrated significant anti-seizure effect in both acute and chronic animal models of seizures (Rogawski et al, 2013). The use of this agent to manage SE in humans, however, has produced conflicting results (Vaitkevicus et al, 2017; Rosenthal et al, 2017; Rossetti 2018) and the agent is not conventionally used in SE management currently.

Learning from the examples of bumetanide and allopregnanolone, therefore, excitement should be tempered when preclinical studies reveal new potential treatment options. Instead, we should continue to exercise patience until high quality clinical data is available.

Concluding remarks

BZP resistance remains a pressing, global clinical problem within the management of SE. Clinical studies show that the duration of SE prior to first treatment is an important factor in determining BZP resistance. This is supported by evidence from animal models, which demonstrate that during persistent seizure activity, GABAergic synaptic transmission alters in multiple ways that can contribute to progressive BZP resistance. Whilst some inconsistencies remain between clinical and experimental studies, evidence suggests that the time since onset of SE should be considered as a critical factor in determining the probability of BZP responsiveness and SE that is prolonged at presentation should lead to very early consideration of adjunctive therapy. An understanding of the cellular and molecular mechanisms underlying BZP-resistance, gleaned from experimental studies, should inform the optimisation of future strategies for managing SE.

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Conflict of Interests

None to declare

Review criteria

The studies mentioned in **Table 1** were found using medical search headings (MeSH) on the PubMed and Embase database search platforms. We searched within the main heading of 'convulsive status epilepticus' and included 'drug therapy' and 'prevention and control' as sub-headings. We added 'benzodiazepines' with the sub-headings 'administration and dosage' and 'therapeutic use' to our search requirements. We limited our search to studies published from 1 January 1990 to 1 July 2021 and only peer-reviewed studies that were published in English and had the full-text available. Studies were included if they were performed on patients, both adult and or paediatric, presenting in CSE and where the efficacy of monotherapy with a BZP (consisting of 1-2 doses), of any kind or formulation, was assessed in terms of its efficacy in terminating SE. In addition to this search, we also assessed the studies mentioned in two systematic reviews[7, 227] and added additional studies that met our inclusion criteria.

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   This seminal multicentre study from a resource-equipped setting focussed on the treatment of adult and paediatric benzodiazepine-resistant status epilepticus.

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