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REVIEW

ATP signalling in epilepsy

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Abstract This paper focuses on a role for ATP neurotransmission and gliotransmission in the pathophysiology of epileptic seizures. ATP along with gap junctions propagates the glial calcium wave, which is an extraneuronal signalling pathway in the central nervous system. Recently astrocyte intercellular calcium waves have been shown to underlie seizures, and conventional antiepileptic drugs have been shown to attenuate these calcium waves. Blocking ATP-mediated gliotransmission, therefore, represents a potential target for antiepileptic drugs. Furthermore, while knowledge of an antiepileptic role for adenosine is not new, a recent study showed that adenosine accumulates from the hydrolysis of accumulated ATP released by astrocytes and is believed to inhibit distant synapses by acting on adenosine receptors. Such a mechanism is consistent with a surround-inhibitory mechanism whose failure would predispose to seizures. Other potential roles for ATP signalling in the initiation and spread of epileptiform discharges may involve synaptic plasticity and coordination of synaptic networks. We conclude by making speculations about future developments.

Keywords Astrocytes · ATP · Epilepsy · Gliotransmission · Seizure

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Abbreviations

AEDs	Antiepileptic drugs
ATP	Adenosine 5′-triphosphate
CNS	Central nervous system
PDS	Paroxysmal depolarisation shift (PDS)

Introduction

Purinergic signalling, defined as adenosine 5′-triphosphate (ATP) released as a transmitter or cotransmitter acting extracellularly on pre- and pos-tjunctional membranes at neuroeffector junctions and synapses, was first described over 35 years ago [1]. Although the idea was initially received with scepticism, purinergic signalling is now widely accepted as many physiological processes incorporate this mechanism [2]. Consequently, purinergic pathophysiology would expectedly play a role in the pathogenesis of disease, thereby giving clues to potential therapeutics. The earliest disease processes believed to incorporate a purinergic basis included pain [3] and migraine [4]. At the present time, a large and growing body of evidence suggests purinergic drug targets may improve a growing list of diseases. For example, clopidogrel, the $P2Y_{12}$ inhibitor, the first of such drugs, is already in use for stroke and thrombosis [5] and, it is hoped, will be a forerunner among many.

Many neurons release ATP as a co-transmitter, and this serves as an activity-dependent signal which evokes a response in astrocytes, Schwann cells and oligodendrocytes that express P2 receptors. Astrocytes can respond by strengthening the synapse, for example by releasing both glutamate and ATP into the synaptic cleft. Schwann cells at the neuromuscular junction respond to axonal ATP release

following an action potential with a rise in intracellular Ca^{2+}. Recognition of a passing action potential is key in the development of oligodendrocyte progenitors into oligodendrocytes [6].

Earlier studies suggestive of a role for purinergic signalling in epilepsy included the finding that seizure-prone mice have increased extracellular ATP levels, possibly owing to decreased brain ATPase activity [7]. Moreover, microinjection of ATP analogues into the rodent prepiriform cortex was shown to cause generalised motor seizures [8]. Thirdly, it was found that P2X7 receptors are upregulated by 80% in hippocampi of pilocarpine-induced chronic epileptic rats, as shown by fluorimetric, immunohistochemical and Western-blotting techniques [9].

Meanwhile, a role for adenosine in epilepsy, particularly status epilepticus, has long been suggested [10]. Recent studies have confirmed such a role and attributed A1 receptor activation to the observed antiepileptic effects [11, 12]. On the other hand, Zeraati et al. [13] elegantly showed that A2A receptors in the CA1 hippocampal region have the opposite effect to A1 receptors in a piriform cortex kindling model.

Adenosine receptors are believed to play a role in presynaptic modulation of neuronal excitability and epileptogenesis [14], although the expression and function of adenosine receptors in various models of epilepsy is controversial. While Angelatou et al. [15] report upregulation of purinergic P1 receptors in the neocortex of patients with temporal lobe epilepsy, other groups report receptor downregulation [16–18]. Purinergic mechanisms in epilepsy have previously been described in the context of adenosine as an antiepileptic agent [19]. Therein, adenosine-releasing grafts have been shown to suppress both seizures [20] and epileptogenesis [21] in kindling models of epilepsy. Furthermore, adenosine kinase inhibition may also promote increased levels of extracellular adenosine thereby providing antiepileptic effects [22].

Indeed, other adenosine-mediated pharmacological strategies are being actively explored, and exciting studies are underway. Additionally, pH variation under respiratory control has been shown to affect cortical excitability in an ATP- and adenosine-dependent manner [23] and this may be relevant to the pathophysiology of epileptic seizures, for example, those in childhood absence epilepsy. In this paper we focus on recent developments in the role of ATP signalling in epilepsy.

ATP as a neurotransmitter and gliotransmitter

ATP released by neurons and glia has several functions including regulation of synaptic transmission both directly and through glia, responses to injury and inflammation, pain, myelination and neurogenesis (for review please see [24]). Additionally, ATP satisfies all of Dale's criteria as a neurotransmitter. Firstly, it is released from synaptosomes in the cerebral cortex, hypothalamus, medulla and other parts of the central nervous system (CNS) with presynaptic localisation in vesicles with high intravesicular concentration [25]. Secondly, upon a physiologically relevant stimulus, ATP is released by a SNARE-dependent mechanism [26]. However, this mechanism is controversial since astrocytic ATP has recently been shown to be released by lysosome exocytosis [27, 28]. Thirdly, it acts with specificity on P2X ligand-gated ion channel receptors and P2Y G-protein-coupled receptors. For a recent review of purinergic receptor expression patterns and functions please see [29]. Finally, ATP incorporates an inactivation system as it is rapidly broken into adenosine by the ectoenzymes ectoATP diphosphohydrolase and ecto-phosphodiesterases/ecto-nucleotide pyrophosphatases [25].

The glial calcium wave

Elevations in intracellular calcium propagate between astroglial cells as the calcium wave [30–32]. This extraneural signal transduction mechanism is crucial to glial homeostasis, and increased intracellular Ca^{2+} leads to a variety of responses including growth, differentiation and release of neuroactive mediators [33, 34]. Calcium waves are believed to be the mechanism of long-distance glial signalling in the CNS as they have been observed to travel more than 500 μm at a velocity of 14 μm/s in culture [35].

Key to the propagation of these calcium waves is ATP released by astrocytes as the calcium wave propagates, and this is facilitated by gap junctions permeable to Ca^{2+} ions [36, 37]. Calcium waves can be evoked by applying ATP, which acts on P2 receptors, the blockade of which attenuates calcium waves [38]. P2Y1 and P2Y2 receptors are both necessary and sufficient for the calcium-wave propagation, and P2Y2 receptors propagate calcium waves faster and further than P2Y1 receptors [39]. In addition, a key role for P2X7 receptors is established as well as possibly contributory roles for P2X2, P2X4, P2X5, P2Y2, P2Y4 and P2Y14 based on agonist preferences [40]. Interesting quantitative models of purinergic junctional transmission of calcium waves have been conducted on astrocyte networks [41, 42].

Astrocytes modulate neurotransmission

Astrocytes have been shown to directly influence neurotransmission at synapses through the release of glutamate [43] and D-serine [44]. The controlled release of

neurotransmitters (or equally, gliotransmitters that modulate neurotransmission) at the synaptic cleft implies and bestows a protagonist role on astrocytes in the tripartite synapse [45].

Early studies indicated that, independent of whether the synapse is excitatory or inhibitory, astrocyte stimulation decreased postsynaptic current responses during synaptic activity [46]. Additionally, miniature postsynaptic current responses were enhanced in frequency but not amplitude [47]. Taken together, these two findings suggest glutamate released by astrocytes modulates presynaptic metabotropic glutamate receptors according to the former finding and NMDA receptors according to the latter. Further studies showed that NMDA receptor-mediated synchrony of neuronal activity may be through modulation of either synaptic [48] or extrasynaptic receptors [49]. Conversely, activity-dependent astrocyte-mediated potentiation of GABAergic synapses has also been demonstrated, suggesting a crucial role in their modulation [50]. Somewhat unintuitively, astrocytic glutamate release is believed to underlie these effects, which are concordant with the findings of Newman and Zahs [51] who first demonstrated neuromodulatory glial control in the retina. Indeed, an interesting recent study has characterised glial neuromodulatory activity down to the level of a single synapse [52].

Furthermore, bidirectional exchange of glutamatergic neurotransmission between astrocytes and hippocampal CA1 pyramidal neurons was elegantly demonstrated in situ [53]. Similarly, Ca^{2+} oscillations in astrocytes have been shown to be induced by neuronal firing [54], suggestive of perisynaptic activation of glial cells. In addition, larger-scale intercellular astrocytic calcium signalling is also believed to be regulated by synaptic activity [55]. Indeed, by virtue of the glial calcium wave, astrocytes have been put forth as important cellular elements involved in the bidirectional processing of synaptic information [56].

All in all, glial calcium waves may either be propagated by "carrying forward" gliotransmission or synaptic activity and may manifest this activation by further influencing neurotransmission or propagating a calcium wave, with a vast array of effects. Gliotransmission combined with glial modulation of neurotransmission is thus suggestive of active bidirectional communication [57]. Calcium waves have therefore been put forth as a mechanism that encodes and transmits information to harmonise neuronal electrical activity, possibly even involving large volumes of activation in the human brain [45]. Therefore, we hypothesize that calcium waves might propagate and therefore synchronise epileptiform activity, as shown in Fig. 1.

The astrocytic basis of epilepsy

Epilepsy is characterised by hypersynchronous neuronal firing although the mechanisms that initiate seizures are largely unknown. As a result, antiepileptic drugs (AEDs)

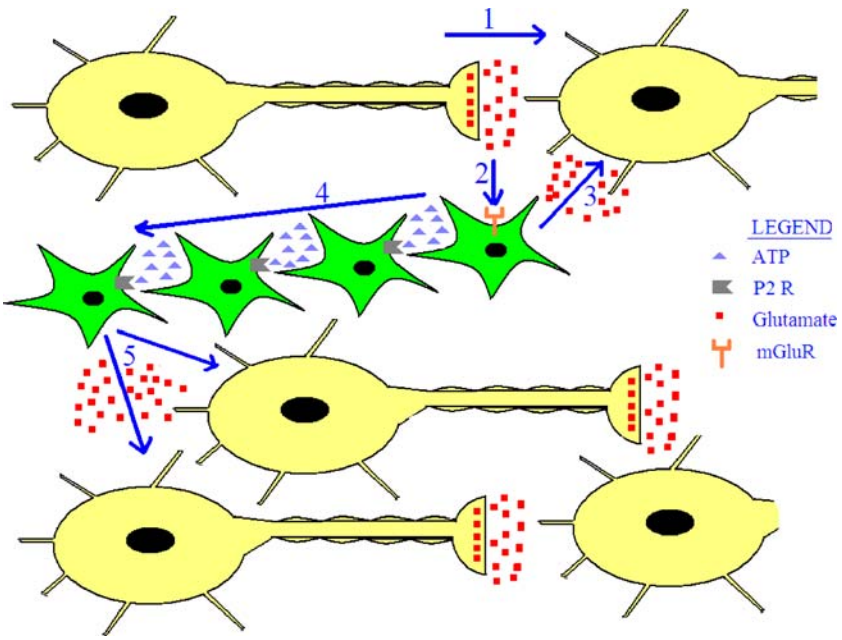

Fig. 1 Calcium wave-mediated synchronisation of neuronal spiking. At a simplified glutamatergic synapse when neurotransmission occurs (*1*), glutamate acts on metabotropic glutamate receptors on astrocytes (*2*), promoting astrocytic glutamate release (*3*) which strengthens the synapse. A parallel activity-dependent calcium wave is propagated by astrocytes releasing ATP, which acts on P2 receptors of adjacent astrocytes (*4*). After the calcium wave has propagated some distance, astrocytes release glutamate at distant neurons and synchronise their spiking (*5*)

simply provide symptomatic relief as they fall into three broad categories—drugs that promote GABAergic neurotransmission (e.g. barbituates, benzodiazepines, vigabatrin and tiagabine); drugs that decrease glutamatergic neurotransmission (e.g. topiramate and felbamate); or drugs that block voltage-gated Na^+ channels (e.g. phenytoin, carbamazepine and lamotrigine) to attenuate high-frequency action potentials in both amplitude and rate of rise [58]. However, in doing so AEDs compromise normal neural function, often with severe side effects [58]. Furthermore, despite a wide variety of AEDs, drug-resistant epilepsy, which is often focal, remains a debilitating problem which often responds only to neurosurgery such as temporal lobe resection in temporal lobe epilepsy, corticectomy or vagus nerve stimulation, which are not without considerable risks and costs.

The cellular correlate of interictal epileptiform activity is the paroxysmal depolarisation shift (PDS)—abnormal prolonged depolarisations with repetitive spiking induced by ionotropic glutamate receptor activation which is thought to drive groups of neurons into hypersynchronous bursting [59]. Astrocytes effect glutamate release upon a Ca^{2+} and SNARE-dependent mechanism [60, 61]. Physiologically, this typically follows increased intracellular Ca^{2+} levels resulting from glial communication via the calcium wave (see below). This phenomenon has been pharmacologically demonstrated by activation of type I metabotropic glutamate receptors (mGluRs) by their agonist dihydroxyphenylglicine (DHPG), by intracellular injection of IP3 or by increasing intracytoplasmic Ca^{2+} levels by photolysis of caged Ca^{2+} [62]. Importantly, glutamate release from astrocytes has been demonstrated to play a key role in the control of synaptic strength and is based upon stimulation of astrocytic P2Y1 receptors based on neuronal activity [63].

The role of this astrocytic glutamate release in triggering PDSs was characterised by Tian et al [64]. Patched CA1 pyramidal neurons from rat hippocampal slices were found to undergo PDSs when exposed to the epileptogenic K^+ channel blocker 4-aminopyridine (4-AP), and the majority (70–90%) of these PDSs were insensitive to TTX, suggesting PDSs can be triggered sans action potentials. Furthermore, photolysis of caged Ca^{2+} in astrocytes but not neurons evoked PDSs in a mechanism consistent with Ca^{2+}-dependent glutamate release. In vivo studies using two-photon imaging of exposed cortex of adult mice during seizures induced by the proepileptic drug 4-AP showed that three AEDs—valproate, gabapentin and phenytoin—decreased 4-AP-induced Ca^{2+} signalling (by 69.7, 55.6 and 45.5% respectively) and ATP-induced Ca^{2+} signalling (by 64.9, 53.8 and 23.8% respectively). A problem with all studies of experimental epilepsy is how closely they match the human disease [65]. This study in particular was controversial as Fellin et al. [66] showed that astrocytic glutamate is not necessary to generate epileptiform activity. Moreover, a recent study has challenged the capability of astrocytes to release glutamate [67, 68].

Notwithstanding, astrocytic glutamate release may explain how glial scarring underlies post-traumatic epilepsy and hippocampal sclerosis leads to mesiotemporal epilepsy. Furthermore, it remains possible that blocking astrocytic glutamate release, for example by inhibiting components of the astrocytic calcium wave, may decrease seizures or prevent their spreading.

Novel antiepileptic drug targets

The mechanism of astrocytic calcium waves is reviewed above, and we propose that this is the initiating event of epileptic seizures because they could theoretically carry-over neuronal excitability from one set of recently activated neurons to another dormant set of neurons. Such a model would spatiotemporally synchronise the second group to fire unexpectedly and in harmonic synergy to the first set which is the neurophysiological hallmark of epileptiform spiking. Secondly, astrocytic calcium-wave signalling mediates interastrocytic excitation, which is the decisive final step prior to astrocytic glutamate release [69], which then acts on neurons to evoke EPSPs [64]. Therefore, we propose that blocking the astrocytic calcium wave represents a proximal, albeit relatively unexplored, drug target for the treatment of focal epilepsy.

From analysis of the models of astrocytic calcium-wave signalling, two obvious drug targets are apparent—gap junctions and P2Y receptors. The role of gap-junction signalling in epilepsy is still unclear [70]. While inhibiting gap-junction signalling has already been shown to have antiepileptic properties [71, 72], there is evidence that ionic conductance through gap junctions only partly accounts for ionic buffering [73]. However, the gap-junction protein Cx43 has been shown to modulate both astrocytic P2Y1 receptor expression levels [74] and pharmacological function [75]. Moreover, ATP efflux from Cx43 hemichannels has recently been demonstrated [76], further expanding the scope of purinergic signalling in gliotransmission. Therefore, the role of gap junctions in propagating the calcium wave, their interplay with P2 receptors, and whether this is the substrate of their antiepileptic effect when inhibited, remains to be fully determined. In the interim, purinergic receptor modulation may hold promise in novel antiepileptic drugs indicated for focal or drug resistant epilepsy.

Moreover, we believe astrocytic purinergic signalling has a more significant role in influencing the synaptic plasticity which perpetuates epilepsy, since ATP is co-released with glutamate in a neuronal activity-dependent manner [77]. We hypothesize that increased extracellular ATP levels promote organisation of neurons into functional

assemblies, especially since ATP has been shown to do the same in development prior to synaptogenesis [78]. This takes prominence since altered synaptic plasticity is believed to lead to neuronal circuits which are strengthened by long-term potentiation-like mechanisms [79], and although preventing synaptic remodelling in epilepsy is a relatively unexplored area, we believe our proposed therapy will decrease long-term remodelling in the epileptic brain. Moreover, since P2 receptor activation is associated with astrogliosis [80], P2 receptor inhibition would be expected to prevent the formation of an epileptogenic focus after brain injury.

Glial calcium waves do, however, represent a complex target owing to a variety of direct and indirect functions. Firstly, calcium-wave signalling underlies glial regulation of cerebral microvasculature and metabolism which may be either proepileptic or antiepileptic [81, 82]. Secondly, calcium-wave signalling may occur either as a cause or an effect of neurotransmission, and a successful antiepileptic strategy would entail targeting only those with a putatively causal role in excitatory neurotransmission. Another source of complexity is the variation in models of calcium-wave signalling (reviewed in [83]), whose underlying mechanisms differ between brain regions. Haas et al. [84] elegantly showed that activity-dependent ATP release propagates within mouse neocortex independent from astrocytic calcium waves, thereby raising the possibility that calcium-wave signalling may have further anatomical variations. However, this is not necessarily a setback as such variation may offer greater specificity in treating different types of seizures as specific anatomical or pharmacological targets are identified.

Taken together, it can be argued that the effects of attenuating glial calcium waves on neuronal networks in the human brain may be hard to predict. However, we argue that the same can be said of inhibiting neuronal firing en masse as a therapeutic strategy in epilepsy. The multitude of functional roles and anatomical variation of gliotransmission is analogous to the nonspecific anatomical and functional variations of neurotransmission (e.g. excitatory versus inhibitory neurotransmission, reductio ad absurdum). In other words, as more is learned about the molecular pathophysiology of the PDS, we simply offer modulation of gliotransmission as an adjunct to inhibiting neurotransmission as a novel antiepileptic approach.

Coordinating synaptic networks

Although ATP acting on P2 receptors is excitatory as it opens cation channels and as it acts on neuronal P2X7 receptors whose clustering in presynaptic densities is suggestive of positively modulating glutamate release [85], astrocyte-released ATP is actually inhibitory to neurons [86]. The mechanism for this is that ATP is rapidly broken down into adenosine by ectonucleotidases (E-NTPDase, E-NPP, alkaline phosphatises, and ecto-5′ nucleotidase; reviewed by Zimmermann [87]), which is inhibitory to neurons [88].

Using an innovative transgenic mouse model expressing a dominant negative SNARE domain to selectively block astrocytic ATP release, Pascual et al. [89] elegantly showed that astrocytic purinergic signalling coordinates synaptic networks. As alluded to above, ATP is released in an activity-dependent mechanism in response to neuronal firing and is hydrolysed extracellularly to adenosine. This accumulated adenosine diffuses and tonically suppresses synaptic transmission at distant sites. The authors suggest that such a mechanism enhances the dynamic range for long-term potentiation and mediated activity-dependent heterosynaptic depression, which then provides a pathway for synaptic crosstalk.

Several issues pertaining to epilepsy arise from this excellent paper. Prima facie, as pathological synaptic transmission underlies epilepsy, awry purinergic signalling is a legitimate possibility. In addition to the authors' description of a role in crosstalk between distant synapses, we propose another role: surround inhibition (Fig. 2).

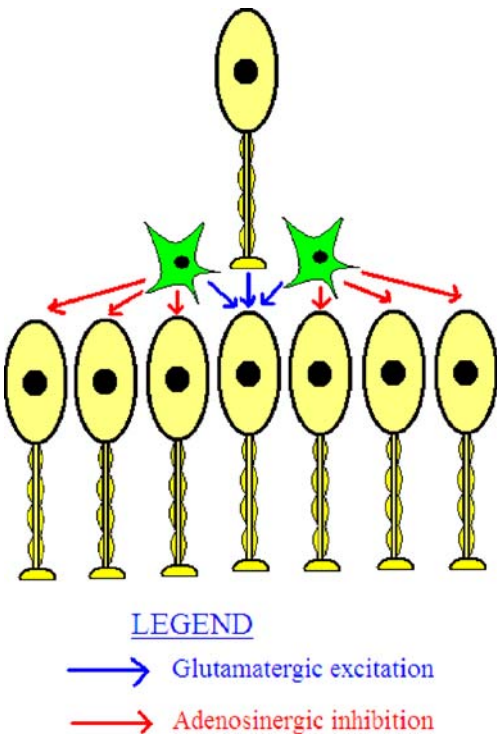

Fig. 2 Activity-dependent ATP release from astrocytes mediates surround inhibition. The synapse in the centre of the figure is strengthened by astrocytic glutamate release. Adjacent neurons are inhibited when ATP released upon neuronal activity is broken down into adenosine

Accumulated adenosine is mediating collateral inhibition of adjacent neurons after neuronal activity, thereby seeking to enhance the signal by reducing background noise.

Reducing this background noise would imply that lower concentrations of glutamate would be necessary for signal transduction, which in turn may prevent neuronal cell death by preventing NMDA receptor overstimulation. A surround inhibitory mechanism, although traditionally ascribed to GABAergic neurons, prevents seizure propagation [90], and its failure would provoke epileptic seizures.

The function of ATP can vary, and it cannot be easily predicted if excitation or inhibition will prevail. While it is possible that there may be a balance, the two functions may also display spatial and temporal separation, as a corollary to Pascual et al. [89]. A temporal separation is more obvious as ectonucleotidases rapidly convert ATP to adenosine. Therefore, any excitatory role of ATP would be immediate and short-acting. Conversely, as the authors conjecture, a spatial separation pattern would emerge secondary to this owing to preferential diffusion of adenosine beyond the synaptic bouton in question leading to a widespread inhibitory function. Based on this model, more ATP may be better in producing more inhibitory adenosine although there is a risk of triggering pathological glial calcium waves [37] during their brief excitatory role.

Other pathological depolarisation phenomena

Two different mechanisms exist that perpetuate secondary injury in the CNS—peri-infarct depolarisations/hypoxic spreading depression [91–93] and glial calcium-wave signalling [37]. Several superficial similarities exist between these two complex phenomena such as the rate of propagation of calcium waves (14 μm/s according to [35]) which is similar to spreading depression (15–35 μm/s) and the fact that both are blocked by purinergic receptor blockers. Therefore, while one paper suggests that astrocyte calcium waves are causal to spreading depression [94], another paper demonstrates that spreading depression can occur in the absence of calcium waves, for example when the bathing fluid is void of calcium [95]. Moreover, the metabolic toxins fluorocitrate [96] and fluoroacetate [97] that poison glia several hours before affecting neurons do not prevent spreading depression, but rather facilitate it (and glia at best play a passive role by attempting to stabilise extracellular K+ levels), bringing us closer to the original theory of spreading depression being a neuronal rather than a glial phenomenon [98]. All the same, there could be as-yet-undiscovered mechanisms that link these two, and until then, separate pharmacological interventions must be devised to address these two phenomena. However, since they both seek to perpetuate secondary injury, purinergic receptor blockade may be neuroprotective in the setting of acute neurotrauma [99, 100].

Conclusion

In summary, first we postulate that blockade of gliotransmission by purinergic modulation may improve epilepsy by decreasing synaptic strength across the tripartite synapse and by preventing synchronous ictal spread to distant sites. Secondly, we hypothesize that ATP released by astrocytes in response to neuronal activity is a source of surround inhibition to adjacent neurons. Such a model would both prevent propagation of seizures and also enhance neurotransmission by decreasing background noise. We hope our opinions are beneficial in the development of treatment strategies for epilepsy and other pathological depolarisation phenomena following neurotrauma.

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