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Organoids as a model of status epilepticus

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ABSTRACT

Status epilepticus (SE) is a neurological emergency that can be studied in animal models, particularly mice. However, these models are labour-intensive and require large numbers of animals, which raises ethical and logistical challenges. Additionally, rodent-based models could lack direct relevance to human physiology. While reduced models offer some insights, they fail to replicate the full complexity of brain connectivity and interactions with other organs. To address this, human forebrain assembloids, formed by both cortical excitatory and subpallial inhibitory neurons, could be an alternative SE model. Assembloids offer a middle ground, enabling high-throughput screening of potential treatments while maintaining relevant human cell biology. This approach could serve as an intermediate step before transitioning to animal models, ultimately reducing the time and number of animals required for SE research. This paper is based on a presentation made at the 9th London-Innsbruck Colloquium on Status Epilepticus and Acute Seizures in April 2024.

1. Introduction

Status epilepticus (SE) is characterized by either a seizure lasting more than five minutes or the occurrence of multiple seizures within a five-minute interval, without a return to normal consciousness between episodes [1]. To study SE, researchers primarily rely on animal models, particularly mammals, which effectively replicate the various stages of this condition, including neuronal damage, associated morbidities, and mortality [2]. These models can be induced electrically or chemically, facilitating investigations into the pathophysiology of SE and the testing of novel therapeutic interventions [2].

While animal models have significantly advanced our understanding and treatment of SE, they possess inherent limitations. Notably, these models lack the human genetic background and fail to capture the diverse aetiologies of human SE, which often arise from various underlying conditions rather than from electrical or chemical insults [2]. Although *in vitro* models face challenges in addressing these complexities, they can leverage 3D human neuron preparations, such as organoids or assembloids, to better reflect the human genetic background and enhance the relevance of research findings.

2. Assembloids to model human forebrain

Status epilepticus is a complex condition and to be modelled would require at least a define 3D structure with physiological network activity [3]. The human cerebral cortex contains both glutamatergic excitatory neurons born in the dorsal forebrain and GABAergic inhibitory neurons born in the ventral forebrain – or subpallium – that then migrate into the dorsal forebrain to meet their excitatory partners [4,5]. Thus, the generation of a physiologically relevant human *in vitro* models of the developing cortex requires the derivation of two different organoid types, specified to the dorsal and ventral forebrain respectively, and their fusion, to recapitulate the migratory pattern of cortical GABAergic interneurons into the dorsal forebrain [6–10]. To date, several protocols have been established to recapitulate interactions between glutamatergic neurons and GABAergic interneurons. These protocols generate a human cortical spheroid (hCS) and a human subpallial spheroid (hSS), which are fused into a human forebrain assembloid (hFA) [6,11,12].

hSSs mainly generate interneurons of medial and caudal ganglionic eminence origin expressing specific inhibitory subtype proteins such as somatostatin, calretinin and calbindin [6]. Parvalbumin is also detected at later stages of development and expressed in hSS after 200 days of maturation. Fusion of hCS and hSS is performed at day 60 of maturation, after which interneurons start to migrate from the hSS into the hCS following the same saltatory mode of migration observed *in vivo* [6].

Following migration from the hSS to the hCS, GABAergic interneurons form synapses with glutamatergic neurons which leads to increased interneuron morphological complexity and integration into a cortical microcircuit [6]. This system is amenable to long-term culture *in*

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vitro and can be probed using a range of functional assays such as live calcium imaging, patch-clamping, local field potential recordings or multi-electrode arrays [3].

In hFAs, spontaneous inhibitory and excitatory post-synaptic currents were recorded in both inhibitory and excitatory neurons on the cortical side after interneuron migration, indicating that cortical interneurons integrate within a cortical microcircuit [6,13]. Importantly, migrated interneurons show increased action potential firing compared to both non-migrated interneurons and interneurons in unfused subpallial spheroids. This aligns with the activity-dependent maturation process that interneurons undergo following their integration in excitatory neuronal networks, which has also been observed in the developing cortex *in vivo* [4]. Network activity has also been assessed using extracellular local field potential recordings in a hFA model, and robust oscillatory activity, ranging from 1 to 100 Hz, in assembloids after 100 days in culture have been shown [14]. Interestingly, there was no measurable oscillatory activity in cortex-cortex fusions, indicating that the presence of cortical interneurons is required for the generation of

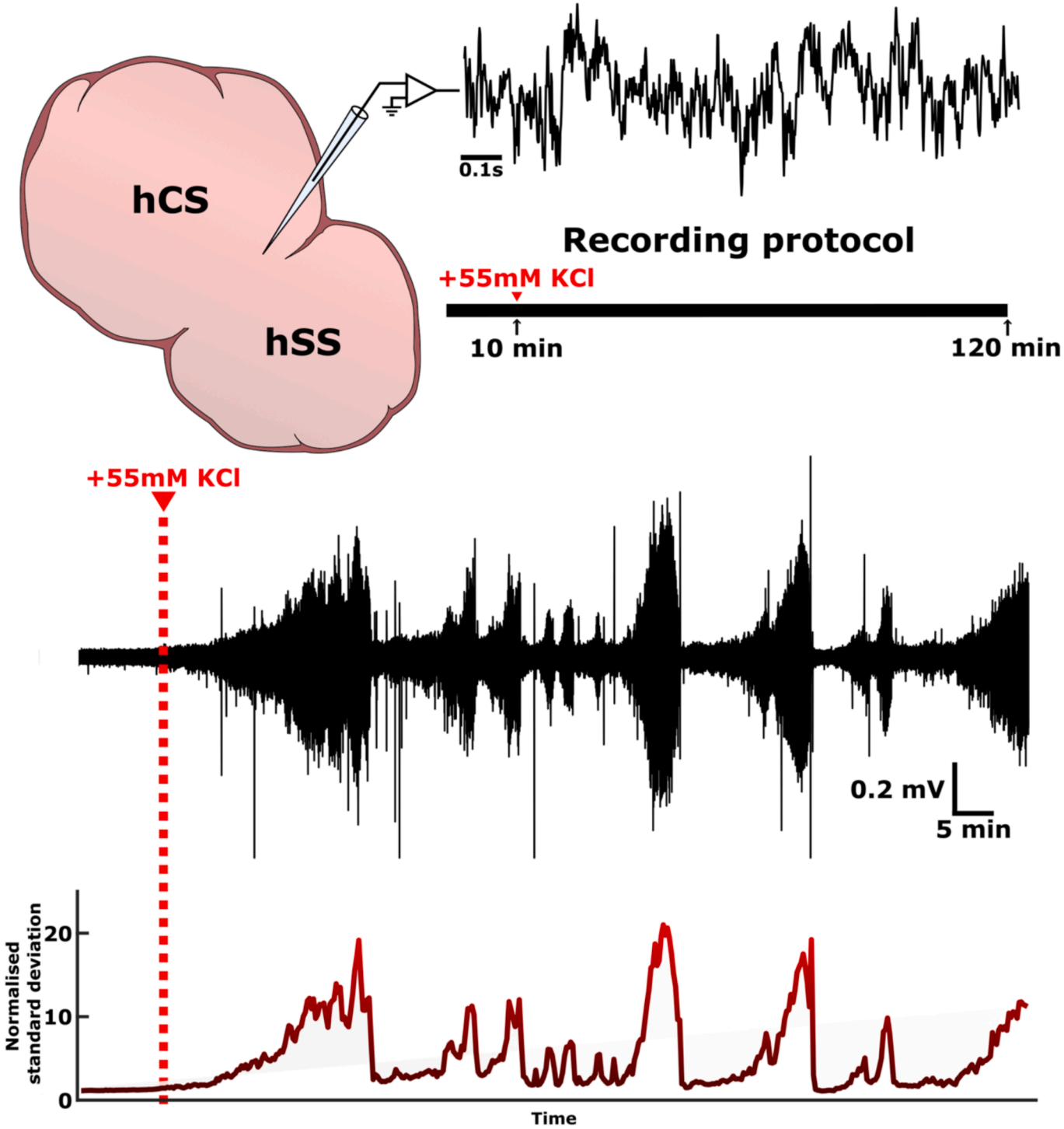

Fig. 1. SE induction in assembloids. Local field potentials (LFPs) from forebrain assembloids recorded at baseline (for 10 min) and in high KCl (+55 mM) perfusion media for more than an hour. After SE induction there was a sustained increase in signal standard deviation compared to baseline. hCS= human Cortical Spheroid; hSS= human Subpallial Spheroid.

higher-order network activities such as multifrequency oscillations [14]. This is in accordance with the emergence of complex oscillatory activity observed after the detection of GABAergic interneurons in cortical and cerebral organoid models, as well as evidence for the regulatory role of interneurons in the oscillatory processes in the rodent cortex [15–17]. Overall human forebrain assembloids provides an *in vitro* model of human neuronal networks that could be studied in the context of epilepsy and SE.

3. Modelling SE in human forebrain assembloids

With the formation of complex networks of neurons, spontaneous neural activity emerges and can be observed in the form of network bursting events and as stable oscillatory dynamics [17,18]. For instance, in 8-month old brain organoids robust theta activity was observed during network bursts, and to which single unit spikes were phase locked [18]. Similarly, fast gamma (100–400 Hz) amplitude, frequently associated with coordinated local unit firing [19,20], has been shown to be significantly phase-amplitude coupled to delta (1–4 Hz) during network bursting events [17].

Forebrain assembloids have been used to model Rett Syndrome, a neurodevelopmental disorder associated with epilepsy [14]. Local Field Potential (LFP) recordings in this model showed signs of epileptiform-like activity characterised by spontaneous events of synchronised calcium transients and high-frequency activity in assembloids derived from Rett syndrome patients, which were not observed in isogenic control assembloids [14]. The oscillatory activity normally observed in this model was also disrupted, with a notable lack of low-frequency and gamma oscillations in Rett Syndrome-derived assembloids.

Importantly, these dynamics can be affected by perturbations made to the environment in which assembloids are grown. Addition of a pro-convulsant, such as potassium chloride (KCl), reliably and rapidly induces epileptiform activity remarkably similar in form to that observed in *ex vivo* acute slice preparations from healthy animals [21].

Recently this method has been used to induce SE in forebrain assembloids [13]. Local field potentials (LFPs) from forebrain assembloids were recorded at baseline (for 10 min) and in high KCl (+55 mM) perfusion media for another hour. After SE induction there was a sustained increase in network activity measured as signal standard deviation changes compared to baseline (Fig. 1).

The remarkable consistency with *in* and *ex vivo* approaches situates forebrain assembloids as a potential tool for *in vitro* screening of novel SE therapies without the use of animals, furthering the ultimate objectives of the 3Rs [22] while remaining translationally relevant through the use of human tissue, often collected directly from patients with the disease.

4. Testing novel SE therapies in human assembloids

Forebrain assembloids have the clear advantage of potential high-throughput screening for SE therapeutics, before testing in animal models.

Recently this *in vitro* SE model has been used to test a novel genetic therapy. The therapy was based on expressing a therapeutic engineered potassium channel (EKC) transgene under the activity-dependent *cfos* promoter [13]. This novel closed-loop therapeutic approach is based on the principle that only hyperactive neurons at a certain point during epilepsy should be treated to rescue the epileptic phenotype, and not the entirety of the neurons all the time such as using constitutive promoters [23]. Forebrain assembloids were transduced with a lentivirus expressing *cfos*-EKC or *cfos*-dsGFP (control), and subsequently incubated in 55 mM KCl for 1 h 45 min in order to increase baseline activity. Four hours later, LFPs from the assembloids at baseline and in high (+55 mM) KCl perfusion media (for 1 h and 40 min) was recorded. Interestingly no major differences were observed at baseline. However, assembloids expressing *cfos*-dsGFP reliably displayed significant increases in LFP power during the entire exposure to KCl, indicative of induced status epilepticus-like episodes. On the other hand, assembloids expressing *cfos*-EKC showed a significant reduction in activity after around 30 min in KCl, indicating that the activity-dependent tool when activated by increased activity was able to counteract the SE activity. These findings in human 3D cultured neurons were consistent with the effect on spontaneous seizures observed *in vivo* in a murine model of temporal lobe epilepsy [13]. To the best of our knowledge this was the first attempt to test a therapeutic agent in human 3D model of SE.

5. Discussion and future direction

High-throughput and human-relevant models of SE are essential for initial efficacy testing of novel therapeutics. While animal models remain necessary due to the intricate nature of SE and its interactions with other organ systems, the timeline for drug testing in these models could be delayed. This approach would not only conserve resources but also reduce the number of animals used in experiments, aligning with the principles outlined by the NC3Rs guidelines.

Assembloids represent a cutting-edge *in vitro* model for studying SE. Although no model can perfectly replicate the complexities of human pathology, assembloids offer numerous advantages over traditional models. Their ability to integrate both excitatory and inhibitory neurons into a functional network, along with their organized cortical architecture, enables more accurate simulations of brain activity. Moreover, the straightforward administration of drugs within these systems enhances their utility for pharmacological studies.

Crucially, the assembloid approach can be further expanded by incorporating additional brain regions [24]. This advancement has the potential to enhance the fidelity of SE models, paving the way for more effective therapeutic development and a deeper understanding of the underlying mechanisms of this condition.

CRediT authorship contribution statement

J.S. Street: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **C. Zourray:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Gabriele Lignani reports financial support was provided by Great Ormond Street Hospital for Children NHS Foundation Trust. Gabriele Lignani reports financial support was provided by UK Research and Innovation. Gabriele Lignani reports financial support was provided by UK Research and Innovation Medical Research Council. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper].

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