Video Article

Preparation of Acute Human Hippocampal Slices for Electrophysiological Recordings

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Abstract

Epilepsy affects about 1% of the world population and leads to a severe decrease in quality of life due to ongoing seizures as well as high risk for sudden death. Despite an abundance of available treatment options, about 30% of patients are drug-resistant. Several novel therapeutics have been developed using animal models, though the rate of drug-resistant patients remains unaltered. One of probable reasons is the lack of translation between rodent models and humans, such as a weak representation of human pharmacoresistance in animal models. Resected human brain tissue as a preclinical evaluation tool has the advantage to bridge this translational gap. Described here is a method for high quality preparation of human hippocampal brain slices and subsequent stable induction of epileptiform activity. The protocol describes the induction of burst activity during application of 8 mM KCl and 4-aminopyridin. This activity is sensitive to established AED lacosamide or novel antiepileptic candidates, such as dimethylethanolamine (DMEA). In addition, the method describes induction of seizure-like events in CA1 of human hippocampal brain slices by reduction of extracellular Mg^{2+} and application of bicuculline, a $GABA_A$ receptor blocker. The experimental set-up can be used to screen potential antiepileptic substances for their effects on epileptiform activity. Furthermore, mechanisms of action postulated for specific compounds can be validated using this approach in human tissue (e.g., using patch-clamp recordings). To conclude, investigation of vital human brain tissue ex vivo (here, resected hippocampus from patients suffering from temporal lobe epilepsy) will improve the current knowledge of physiological and pathological mechanisms in the human brain.

Introduction

Epilepsy is one of the most common neurological disorders, affecting 1% of the world population, and is associated with increased morbidity and mortality[1,2]. Unfortunately, one-third of patients suffering from epilepsy are drug-resistant, despite an abundance of available treatment options including more than 20 approved antiepileptic drugs (AEDs)[3]. Failure to translate results from preclinical animal research to clinical trials is one reason why promising treatment strategies are not effective in many patients[4]. Recently, neuropeptide Y (NPY) and galanin have been shown to have antiepileptic effects in animal models; though, when tested in resected human brain tissue, only NPY was effective[5].

Most of the existing knowledge concerning basic neurological mechanisms and disease therapy approaches stem from animal models and cell culture experiments. Although informative, these models only represent single aspects of complex human diseases and the adult human brain network. Alternatively, human brain tissue has the potential to bridge the translational gap but is rarely available for functional studies. For instance, post mortem brain tissue has been a valuable tool in investigating protein expression, brain morphology, or anatomical connections, though neuronal activity is often compromised is this tissue[6,7,8,9,10,11].

In contrast, living resected human brain tissue has been investigated concerning preclinical drug evaluation, basic neuronal functions and gene expression patterns[12,13,14,15,16,17]. A great advantage of human brain slices compared to rodent slices is the long viability of neuronal tissue after resection and preparation. Compared to rodent brain slices, which can typically be recorded for up to 8 h after preparation, human brain slices show stable neuronal activity for up to 72 h, enabling thorough investigation of these rare and valuable samples[12,18].

Several studies have investigated properties of epileptiform activity in various areas of resected cortical and hippocampal human tissue and used different methods for induction of epileptiform activity. In rodent slices, epileptiform activity can be induced by several methods: electrical stimulation of DG hilar cells, increase of extracellular K^+ (8–12 mM KCl), blocking of $GABA_A$ receptors by bicuculline (BIC), blocking of potassium channels by 4-aminopyridine (4-AP), and removing or reducing Mg^{2+} in extracellular solution[19]. However, induction of epileptiform activity in human tissue requires the combination of at least two of the abovementioned methods[20,21,22].

Presented here is a method for the preparation of human hippocampal brain slices, which are viable for up to 20 h and show induction of epileptiform activity upon application of high K^+ (8 mM) and 4-AP or low Mg^{2+} and BIC.

Protocol

Patients must give informed written consent prior to operation, and necessary ethical agreements must be in place prior to the experiment. Concerning the representative results, all studies involving human participants were reviewed and approved by Charité-Universitätsmedizin, Berlin (EA2/111/14).

1. Preparation of 10x solutions

NOTE: Due to difficulties in planning access to human brain tissue, it is recommended to prepare 10x solutions as described here. Alternatively, final 1x solutions can be prepared freshly by adding individual substances in final concentration to double-distilled water (ddH_2O).

1. For individual 10x solutions, add substances to ddH_2O according to **Table 1** and stir until dissolved.
2. Use 10x solutions up to 1 month after preparation (up to 1 year for frozen 10x choline aCSF).
3. For 10x choline aCSF, prepare 50 mL aliquots of 10x 1.1 choline aCSF (**Table 1**) and freeze at -20 °C or -80 °C until further use.
   NOTE: Do not add glucose and $CaCl_2$ to 10x 1.1 choline aCSF to prevent contamination with bacteria and precipitation of calcium carbonate.
4. 10x solution 2 can be used for all 1x final solutions, whereas 10x solutions 1.1–1.4 are customized and named accordingly (**Table 1**).

2. Preparation of 1x final solutions

NOTE: Final 1x solutions should be prepared fresh or earliest as possible on the day before use. All final solutions should be carbogenated with 5% CO_2 and 95% O_2 using a glass gas disperser to enrich solutions with oxygen, and adjust the pH to 7.4 (max = 7.4 ± 0.2).

1. Choline aCSF for transport and preparation
   1. For the final 500 mL solution, thaw a 50 mL aliquot of the 10x solution 1.1 aliquot for choline aCSF in 37 °C water bath.
   2. Add the thawed 50 mL aliquot of the 10x solution 1.1 and 50 mL of 10x solution 2 to approximately 300 mL of ddH_2O.
   3. Add final concentrations of glucose and $CaCl_2$, then stir until dissolved (**Table 1**, solution 1.1).
   4. Add ddH_2O to a final volume of 500 mL and measure osmolarity (300 mOsm ± 10 mOsm).
   5. Optionally, use a filter to sterilize the solution (see discussion on prolonged slice viability under sterile conditions).
   6. Fill a separate bottle with approximately 100 mL of 1x choline aCSF for transport from the operation room to laboratory.
   7. Optional: depending on the transport time from the operation room to laboratory, consider using gas-tight bottle caps to ensure stable pH of aCSF during longer transportation periods.
   8. Store the final solution at 4–8°C until further use.
   9. On the day of operation, chill 1x choline aCSF on ice and carbogenate for at least 10–15 min using a glass gas disperser connected to carbogen gas (5% CO_2, 95% O_2).
      NOTE: Consider keeping a gas bottle accessible to the operation room in the case of longer waiting times, which will require re-carbogenation of the transport solution. However, we have transported hippocampal tissue without re-carbogenation before long and short transportation times (15 min vs. 60 min) and did not observe differences in induction of epileptiform activity.

2. aCSF for storage and recording
   1. For a final 2 L solution, add 200 mL of 10x solution 1.2 (aCSF) and 200 mL of 10x solution 2 and glucose (**Table 1**) to ~1500 mL of ddH_2O.
      NOTE: Volumes of the final solutions depend on applied experiments and the type of chamber used for storing and recording.
   2. Add ddH_2O to a final volume of 2 L and measure osmolarity (300 mOsm ±10 mOsm).
   3. Prewarm the solution to 35 °C and carbogenate for at least 10–15 min before use.

3. HighK^++4-AP aCSF for induction of burst activity
   1. For a final 1 L solution, add 100 mL of 10x solution 1.3 (highK^++4-AP aCSF) and 100 mL of 10x solution 2 to ~700 mL of ddH_2O.
   2. Add glucose and 4-AP (final concentration = 100 µM) according to **Table 1**.
   3. Add ddH_2O to final volume of 1 L and measure osmolarity (300 mOsm ±10 mOsm).
   4. Prewarm the solution to 35 °C and carbogenate for at least 10–15 min before use.

4. LowMg^{2+}+BIC aCSF for induction of seizure-like events (SLEs)
   1. For a final 1 L solution, add 100 mL of 10x solution 1.4 (lowMg^{2+}+BIC aCSF) and 100 mL of 10x solution 2 to ~700 mL of ddH_2O.
   2. Add glucose and BIC (final concentration = 10 µM) according to **Table 1**.
   3. Add ddH_2O to final volume of 1 L and measure osmolarity (300 mOsm ± 10 mOsm).
   4. Prewarm the solution to 35 °C and carbogenate for at least 10–15 min before use.

3. Preparation of interface chamber

1. In an interface chamber, slices rest on three layers of filter paper to ensure sufficient amount of solution below the slice. To do this, cut two ~4 cm x ~2 cm pieces of filter paper for each slice-holding compartment (the described interface chamber consists of two compartments) and place them on top of each other.
2. Place thin cotton strings around the 4 cm x 2 cm filter papers inside the compartments to break the tension of the solution. Ensure even flow (here, black nylon tights cut to thin-cut strings ~10 cm in length are used; for placement, see **Figure 1**).

3. Place small pieces of filter paper on top of the larger filter papers inside the slice-holding compartments. Small filter tissue pieces should be roughly the size of one brain slice (~1.5 cm x ~1.0 cm) and will enable further handling of individual slices. Place three to four small filter paper pieces in each compartment.
4. Ensure an aCSF flow rate of 1.8 mL/min with a peristaltic pump.
5. Carbogenate and prewarm the interface chamber to ~35 °C (final temperature of the slice should be ~32 °C).

4. Set-up of preparation area

NOTE: Preparation can be performed under sterile conditions to avoid contamination and elongate slice survival. However, not all vibratomes fit under a sterile hood, and other measures are required to reduce contamination during preparation. This section describes some of these measures.

1. Wipe the preparation area with 70% EtOH and place either aluminum foil or sterile covers on top of the area.
2. Prepare super glue, two sharp tweezers, a spatula, a scalpel with blades, and a blade for rough cutting of the brain tissue. Tools can be sterilized prior to the procedure to reduce contamination.
3. Wipe the buffer tray and specimen plate of the vibratome with 70% EtOH. Once the buffer tray is fully dry, cover it with aluminum foil and place the tray in the ice bath. Fill the ice bath with crushed ice and keep at -20 °C until preparation.
4. Wipe the vibratome and razor blade with 70% EtOH and calibrate the vibratome to minimize vertical vibrations and tissue damage during slicing procedure.

5. Tissue slicing and storing

1. Directly after resection, place the tissue immediately in cold, carbogenated choline aCSF and transport quickly to the laboratory.
   CAUTION: Wear gloves and a face mask at all times during preparation, since human brain tissue can contain potential pathogens. In addition, wearing a face mask when not working under a sterile hood will greatly reduce contamination of solutions and brain tissue.
2. Remove tissue from choline aCSF and cut away any burned portions of tissue.
3. Cut an even surface to glue tissue piece onto the specimen plate, while considering the cutting angle and tissue layers. Ideally, a hippocampal slice contains DG, CA1-4, and (if possible) subiculum.
4. Slice the brain tissue into 400 µm thick slices and adjust the amplitude and speed during cutting. Due to possible remaining pia mater, human brain tissue shows more resistance and may require slower cutting.
   NOTE: Slice thickness greatly affects either the available network (more neurons in thicker slices) or viability of the slice (penetration of solution into the slice). We have used 500 µm slices to increase the potentially available micro-network, and could not observe differences in induction of epileptiform activity. 300 µm slices are commonly used for patch-clamp experiments, though the induction of epileptiform activity in these slices have not yet been tested here. We use 400 µm as a standard slice thickness, though 300–500 µm slices may be sufficient.
5. Before collecting, use a scalpel to reduce the sizes of brain slices to fit into the recording chamber. For use of the membrane chamber (see section 6), slices should be maximally 1.5 cm x 1 cm. While reducing, consider the specific layers and connections needed to be intact for recording (e.g., for recording in the CA1 and DG, cut away the subiculum and surrounding white tissue).
6. Using a spatula and small forceps, carefully place slices in the interface chamber on small filter papers and let them rest for ~1 h in aCSF until recording.
7. Slices can be recorded for up to 20 h (even longer when under sterile conditions).

6. Recording of epileptiform activity

1. In the membrane chamber (submerged type recording chamber), place the brain slice on a transparent semipermeable membrane, which is glued to a plastic ring[24]. For this, use super glue to attach the plastic ring to the membrane of a cell culture insert.
2. Use a scalpel to remove any membrane on the outside of the plastic ring. Ensure that the membrane is even and fully attached to the ring before placing the membrane in the chamber.
   NOTE: The membrane can be stored in ddH_2O at 4–8 °C and reused for up to 1 month. Keep the membrane wet at all times.
3. Both the inflow and outflow of the membrane chamber are connected to tubes for solution supply. Place the tubes in a peristaltic pump so that the inflow and outflow move in opposite directions.
4. Place the inflow and outflow tube in carbogenated, prewarmed aCSF until all tubes and the chamber are filled with solution. Adjust the speed of the peristaltic pump to achieve an even flow rate of 10–13 mL/min.
   NOTE: The membrane chamber used here is a high flow rate, submerged type recording chamber enabling a solution flow of up to 14 mL/min[24]. In the case of using a different submerged type recording chamber, flow rates need to be adjusted. However, for induction of epileptiform activity, it is highly recommended to use the membrane chamber.
5. Use a heating element connected to the inflow in close proximity to the membrane chamber to ensure a stable temperature of 32 °C.
6. Prepare 1–2 MΩ glass pipettes using a vertical puller. Fill pipettes with 154 mM NaCl solution and place them in an electrode holder.
7. Using tweezers and a spatula, remove a hippocampal slice from the interface chamber by taking the slice with the small filter paper and placing both in a Petri dish filled with carbogenated aCSF. Remove the small filter paper from the hippocampal slice, and (if necessary) apply some force using a pipette to separate the slice from the filter paper. Be careful not to flip the slice.
8. Place the slice in the recording chamber and hold it in place using slice mesh.
   NOTE: Due to Bernoulli's principle, in the used submerged type membrane chamber, slices are usually stable without use of an additional slice mesh.
9. Place electrodes in the region and layer of interest (here, CA1) and begin recording.
10. Record field potential activity in current clamp mode with a sampling rate of 10–20 kHz and low-pass filtered at 2 kHz.
11. Record basal activity in aCSF for up to 5 min.
12. Switch the inflow tubes from aCSF to highK^++4AP or lowMg^{2+}+BIC aCSF and the outflow tube to a waste container to prevent mixing of solutions. After 2 min, place the outflow tube in the same solution as the inflow to conserve solution.

13. Burst activity induced by highK^++4-AP should be visible 2–5 min after the wash in. However, induction of SLEs by lowMg^{2+}+BIC can take up to 30 min. If necessary, change the positions of electrodes carefully to obtain optimal results.
14. Once in the final position, record baseline activity for at least 20 min. If you record SLEs, consider longer baseline recordings due to low frequency of SLEs.
15. In the case that baseline activity is stable (plateau of event frequency), wash in the desired drug. Note that due to the high flow rate wash in of drugs takes only 2–5 min, allowing for fast solution exchange.
16. Record activity during drug application of at least 20 min, following wash out. Activity should be stable for at least 60–90 min, allowing for longer recordings.

7. Analysis

1. Analysis of frequency and amplitude can be performed with any available software. So far, we have not been able to establish a reliable automatic analysis of SLEs or burst activity, and instead used a semi-automated analysis with visual confirmation of identified activity.
2. Burst activity is characterized by biphasic, positive, and negative deflection and a duration of ≥100 ms. All events visually identified as burst activity (e.g., semi-automatically by threshold analysis) should be manually indicated for further analysis of event frequency (inter-event interval, IEI), amplitude, and total number of events during the analyzed time frame.
   NOTE: Due to the high frequency of burst activity, the last 5 min of each application phase is typically analyzed[20].
3. SLEs can be analyzed as described in Heuzeroth et al. Identified SLEs can be further analyzed for duration, amplitude, spike frequency, and duration of tonic (high frequency spiking) vs. clonic (low frequency spiking) phase duration. SLEs that are <10 s in duration should be excluded from analysis.
   NOTE: For preclinical evaluation of possible antiepileptic substances, effects on burst activity (induced by highK^++4AP) are being investigated, due to established induction in resected hippocampal tissue. Preliminary results on induction of SLEs using lowMg^{2+}+BIC have been reported (**Figure 2**), though analysis of this data is not included here.

Representative Results

Epileptiform activity has been successfully recorded in resected human hippocampal tissue originating from up to 15 patients. Establishing stable transport and preparation procedures is critical for successful induction of epileptiform activity in human brain tissue. Recently published results have shown 1) stable induction of epileptiform activity in resected tissue of different patients as well as 2) the use of resected human brain tissue as a preclinical tool for evaluation of novel antiepileptic mechanisms[14,20].

Application of highK^++4-AP induced epileptiform activity in form of burst activity within a few minutes (**Figure 2A,B,C,D**). Due to low neuronal distribution in human hippocampal tissue or high neuronal cell loss due to temporal lobe epilepsy (TLE), placement of electrodes can be adjusted in the beginning of recording. In cases where burst activity of slices is not visible in the CA1 area after 10 min (independent of electrode placement), slice viability may be compromised, and the slice will need to be replaced.

SLEs, with a duration of >10 s, can be induced with application of lowMg^{2+}+BIC (**Figure 2E**,**F**). **Figure 2E** shows stable induction of SLEs after a few minutes and stable frequency throughout the recording. Here, SLE activity was successfully induced in two of four slices from the investigated patient. One slice showed only burst activity after 15 min of SLE activity, whereas the other slice did not show SLEs even after 40 min.

For preclinical evaluation of substance effects, a potential antiepileptic effect on burst activity induced by highK^++4-AP was investigated. Known and potential antiepileptic substances (lacosamide, DMEA, dynorphine[14]) were tested, and examples are shown here for the conventional AED lacosamide (a sodium channel blocker) as well as DMEA (a novel potential antiepileptic substance)[20]. The number of events and inter-event interval (IEI) of burst events decreased both during application of lacosamide and DMEA (**Figure 3C**), though amplitudes were mostly unaffected (**Figure 3D**). In a subset of slices, even though induction of burst events was achieved in the first few minutes, the frequency of activity did not recover during wash out of applied AEDs (data not shown here, see Kraus et al.[20]). Here, the applied drugs were considered to induce effects; however, decreases in burst activity may have been affected by the gradual decay in activity during long recordings. Thus, results must be interpreted carefully.

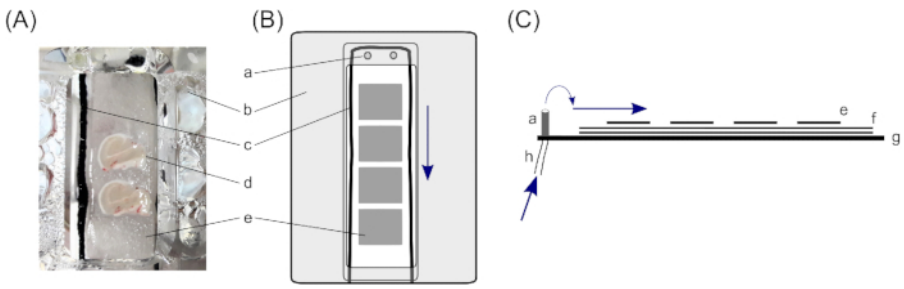

Figure 1: Interface chamber. For storing of human hippocampal brain slices, an interface chamber with two brain slice holding compartments is used (**A**); specifically, a Haas-type interface chamber[23]. Here, hippocampal brain slices rest on (**d**) three layers of filter paper, (**e**) smaller pieces to enable handling of individual brain slices, and (**f**) bigger filter paper pieces to ensure a sufficient layer of solution below the slice. (**c**) A cotton string surrounding the brain slices, on top of the filter papers, ensures even solution flow from the inlets at the (**a**) top of the compartment. (**b**) A cover lid directs oxygen from below the compartment onto the slice. **(B)** Top view of one slice-holding compartment. **(C)** Side view to illustrate the layers of filter papers. **(g**) Bottom of the chamber. (**h**) Tube for solution inflow, which is connected to a peristaltic pump (blue arrows mark the direction of the solution flow). Please click here to view a larger version of this figure.

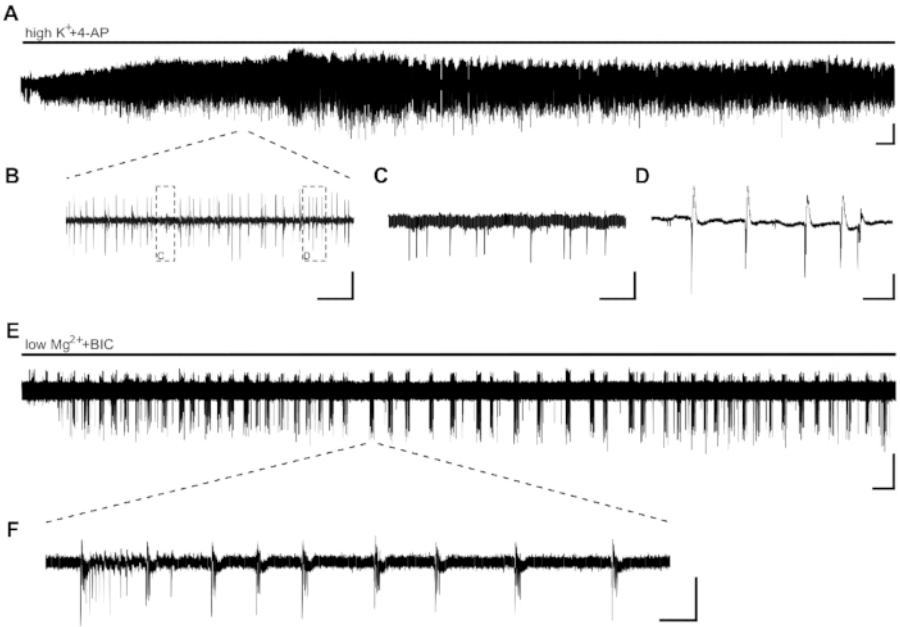

Figure 2: Epileptiform activity in human hippocampal slices induced by highK^++4-AP and lowMg^{2+}+BIC. CA1 example recordings and excerpts of application of highK^+ (8 mM)+4-AP (100 µM) **(A,B,C,D)** and lowMg^{2+}+BIC (10 µM) **(E,F)**. **(A)** Bath application of highK^++4-AP induces epileptiform activity within a few minutes, and activity is stable for at least 60 min. Details of (A) can be seen in (B). Two different types of activity are induced in the CA1 area of human hippocampal slices: interictal-like spikes **(C,** details of [B]**)** and burst activity **(D,** details of [B]**)**. Burst activity was shown to be sensitive to antiepileptic drugs and therefore analyzed for the effect of potential antiepileptic substances (**Figure 3**). **(E,F)** Application of lowMg^{2+}+BIC induces SLEs at a duration of >10 s (**F**) in CA1 within a few minutes. However, induction of SLEs can take up to 30 min in other slices. Scale bars = 0.2 mV, 2 min (A,E), 5 s (B), 500 ms (C,D), 5 min (E), and 2 s (F). This figure has been adapted from Kraus et al.[20]. Please click here to view a larger version of this figure.

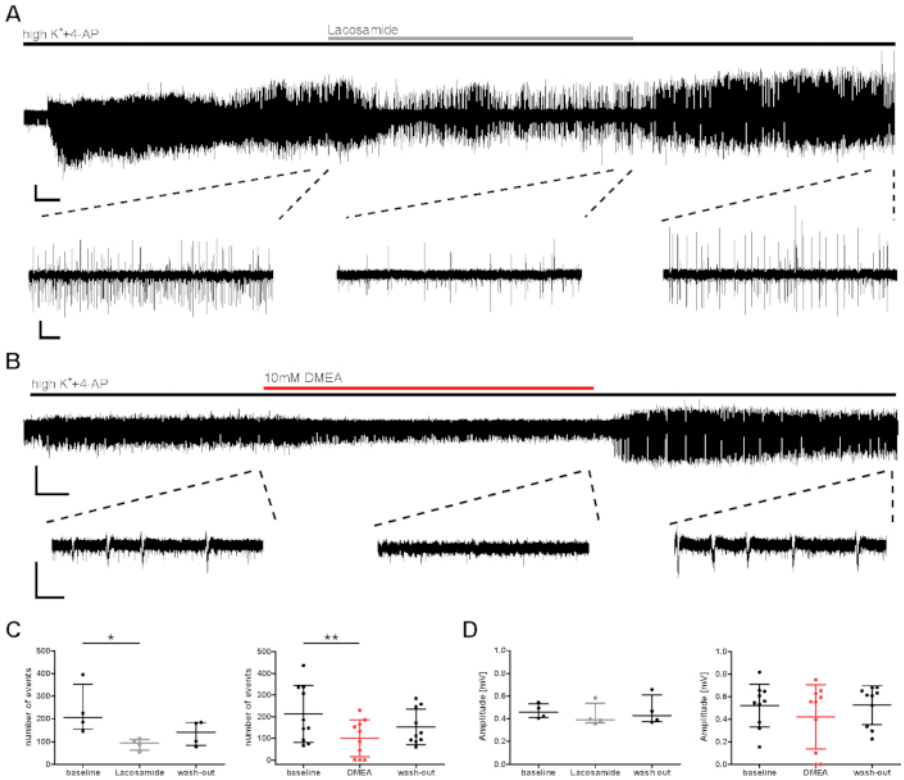

Figure 3: Decrease in epileptic burst activity of human slices during application of lacosamide or DMEA. Burst activity decreased during application of (**A**) lacosamide and (**B**) DMEA, a potential new antiepileptic molecule. (A) and (B) show exemplary recordings of the CA1 area with excerpts of regions used for analysis in (C) and (D). Burst activity decreased during lacosamide (100 µM) and DMEA (10 mM) application, as seen by middle excerpts and increases again during wash out. **(C,D)** Number and amplitude of burst activity were analyzed for the last 5 min of each application phase (baseline, lacosamide/DMEA, wash out) and shown as summarized results for all patients (number of events, C; amplitude, D) as mean ± SD. Each dot indicates one patient. Asterisks mark significant differences as assessed by either Friedman test and post-hoc with Dunnett's multiple comparison of groups for analysis of lacosamide application (*$p < 0.05$, $n = 4$) or by repeated measurement ANOVA and post-hoc with Tukey's comparison for analysis of DMEA application (**$p < 0.01$, $n = 10$). Scale bars = 0.2 mV, 2 min (full recording, A), 5 s (excerpts, A), 3 min (full recording, B), and 1 s (excerpts, B). This figure has been adapted from Kraus et al.[20]. Please click here to view a larger version of this figure.

Solution 1.1 choline aCSF			
Substance	10x concentration (mM)	1x concentration (mM)	Note
choline Cl	1100	110	
(+)-Na L-ascorbate	116	11.6	
MgCl2x6H2O	70	7	
Na pyruvate	31	3.1	
KCl	25	2.5	
NaH2PO4	12.5	1.25	
NaHCO3	260	26	
CaCl2	-	0.5	add to final solution
Glucose	-	10	add to final solution
Solution 1.2 aCSF			
Substance	10x concentration (mM)	1x concentration (mM)	Note
NaCl	1290	129	
NaH2PO4	12.5	1.25	
CaCl2	16	1.6	
KCl	30	3	
MgSO4	18	1.8	
Glucose	-	10	add to final solution
Solution 1.3 highK^++4-AP aCSF			
Substance	10x concentration (mM)	1x concentration (mM)	Note
NaCl	1240	124	
NaH2PO4	12.5	1.25	
CaCl2	16	1.6	
KCl	80	8	
MgSO4	18	1.8	
Glucose	-	10	add to final solution
4-AP	-	0.1	add to final solution
Solution 1.4 low$Mg2^+$+BIC aCSF			
Substance	10x concentration (mM)	1x concentration (mM)	Note
NaCl	1300	130	
NaH2PO4	12.5	1.25	
CaCl2	16	1.6	
KCl	30	3	
Glucose	-	10	add to final solution
BIC	-	0.01	add to final solution
Solution 2			
Substance	10x concentration (mM)	1x concentration (mM)	Note
NaHCO3	210	21	

Table 1: Preparation of 10x and final 1x solutions for transport, preparation, and recording.

Discussion

Living resected human brain tissue is a highly valuable tool in preclinical evaluation of AEDs, as it properly represents an intact human brain micro-network. The presented protocol describes a method for tissue transport and preparation, which ensures high quality hippocampal slices as well as a stable induction method for epileptiform activity critical for AED evaluation.

Investigation of epileptiform activity as well as methods for chemical or electrical induction in human brain slices have been previously shown by other groups[17,20,21,22]. This protocol describes the induction of stable burst activity in slices from different patients via application of high K^++4-AP as well as induction of SLEs in CA1 area via application of low Mg^{2+}+BIC. It was found that the induction of burst activity is more consistent (80% of tested slices in 15 patients) than the induction of SLEs (50% of tested slices in one patient). However, thus far, the induction of SLEs has only been testd in one patient. Nevertheless, induction of SLEs by low Mg^{2+}+BIC is recommended, as SLEs have not yet been able to be induced using high K^++4-AP.

Several studies have introduced methods for transport and preparation of human brain tissue and often highlight three factors critical to neuronal survival: transportation time, used transport solutions, and storing conditions.

For optimal slice viability, some groups suggest that the transport of resected brain tissue be as short as possible. However, operation rooms and laboratories are rarely in close proximity, meaning that slice quality may be compromised due to long transportation. Some groups have overcome this obstacle by applying constant O_2 to the solution during transport[12]. We have transported brain tissue for short (max = 15 min) and long (up to 1 h) periods of time without constant additional O_2 supply during transport, similar to other groups[18,25]. In these cases, differences in tissue quality were not observed during epileptiform recordings. In communication with other groups at our institute, slice quality did not change for patch-clamp experiments, either. In contrast, variance in tissue quality possibly stems from damage during operations, prolonged resection, and slicing procedure.

Concerning transport and cutting solution, all published methods omit NaCl from solutions to reduce cell swelling due to osmotic pressure, similar to the standard procedure for rodent patch-clamp experiments. However, several substitutes have been introduced so far (i.e., sucrose based aCSF[13,22], NMDG-based aCSF[12,26], and choline-based aCSF[27]). Ting and colleagues introduced the NMDG-based aCSF for slice preparation in 2014[26] and later added a recovery protocol, which slowly reintroduces NaCl to the slices[28]. However, as described by Ting et al., neurons of brain tissue prepared in NMDG-based aCSF show higher membrane resistance, thus affecting whole-cell seal during patch-clamp experiments[26]. Therefore, we have transitioned from NMDG-based aCSF to the use of choline-based aCSF[20], which yields high quality slices for both field potential and patch-clamp recordings.

Concerning storage of slices, it is generally accepted that interface conditions provide optimal oxygenation critical for long slice survival[18]. However, other groups show slice survival for up to 72 h under submerged conditions[12]. Contrary to previous hypothesis, human brain slices seem to be more resistant to low oxygenation or oxidative stress compared to rodent slices. Primarily, interface chambers have been previously used for storing of human hippocampal slices, though submerged conditions are recommended for the maintenance of human brain slices in patch-clamp experiments.

As discussed by other groups, an additional critical step for long slice survival (interface for <48 h[18], submerged for <72 h[12]) is the prevention of bacterial contamination. Rodent brain slices are typically used in electrophysiological recordings for up to 8 h, and bacterial contamination is not considered to affect slice viability during this period. High number of slices prepared from one resection and the uncommon availability of human brain tissue highlights the need to prolong viability of human brain slices. This method successfully describes the preparation of living human hippocampal brain slices, which can easily be adapted to sterile conditions. However, for the recordings performed here, slice survival extending 20 h was not a priority.

Recording in interface chambers has also been shown to be essential for induction of epileptiform activity such as SLEs[22]. Submerged conditions, due to low oxygenation, are rarely used for recording of SLEs; though, they are necessary for optical high resolution needed for patch-clamp experiments. The use of an optimized submerged type recording chamber enables the recording of epileptiform activity (extracellular field or single neuron) in human brain slices, due to high oxygenation and fast drug application[29]. Here, methods and results for field potential recordings are described, but it should be emphasized that patch-clamp recordings have been successfully performed in mouse and human brain slices using this modified recording chamber (data not shown).

Resected human brain tissue has a higher translational value compared to rodent models. It represents an adult, diseased neuronal network that cannot be reproduced by iPSCs. However, as in any *in vitro* system, human brain slices do not represent an intact human brain. Additionally, the recorded neuronal networks of resected brain tissue can undergo substantial molecular and functional changes due to damage during operation or preparation. Slicing procedures have been shown to affect GABAergic function and may affect the induction of epileptiform activity[30]. These limitations should be considered while formulating a hypothesis. When testing potential antiepileptic drugs, the use of different brain areas should be considered, as drug targets might not be expressed in all human brain regions or all patients. In particular, the hippocampi of TLE patients often show signs of hippocampal sclerosis accompanied by severe neuronal cell loss. It is recommended to obtain patient information on pathological changes and disease history, such as potential refractory towards medications, and consider this during data interpretation.

In conclusion, this method successfully describes the preparation of living human hippocampal brain slices and induction techniques for recording two different types of epileptiform activity. Since the availability of living human brain tissue is rare, optimized transport and recording conditions should be used to ensure maximum output from experiments using human brain slices. It is suggested that resected human brain tissue can be used as a preclinical validation tool in addition to rodent models and cell culture experiments.

Disclosures

The authors declare no conflict of interest.

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