Journal of Visualized Experiments

Preparation of acute human hippocampal slices for electrophysiological recordings

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE61085R1
Full Title:	Preparation of acute human hippocampal slices for electrophysiological recordings
Section/Category:	JoVE Neuroscience
Keywords:	Electrophysiology; epilepsy; Drug development; Human; hippocampus; ex vivo
Corresponding Author:	Larissa Kraus Charite Universitatsmedizin Berlin Berlin, GERMANY
Corresponding Author's Institution:	Charite Universitatsmedizin Berlin
Corresponding Author E-Mail:	larissa.kraus@charite.de
Order of Authors:	Larissa Kraus
	Laura Monni
	Ulf C. Schneider
	Julia Onken
	Philipp Spindler
	Martin Holtkamp
	Pawel Fidzinski
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Open Access (US$4,200)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Berlin, Berlin, Germany

Aaron Berard, PhD
Editor-in-Chief
JoVE- Journal of visualized experiments 03.12.2019

Dear Dr Berard,

Thank you for inviting us to submit a manuscript to JoVE.
We would like to submit our manuscript entitled “Preparation of acute human hippocampal slices for electrophysiological recordings” for publication as an open access article produced by JoVE.
Our work describes a method for transport and preparation of resected human hippocampal brain slices, which show electrophysiological field potentials for up to 20h. In addition, we introduce two different methods for induction of burst activity (considered interictal) and seizure-like events (considered ictal) in the CA1 region of human hippocampal brain slices.
Our protocol and experimental setup presents a highly valuable tool for preclinical evaluation of antiepileptic drugs in living resected human brain tissue.
We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript and agree with submission to JoVe.
The authors had the following contributions:
LK and PF designed the study, MH selected patients for operation, UCS, JO and PS informed and operated patients, LK and LM performed recordings in human tissue, LK analyzed all data, LK and PF wrote the manuscript, which all authors edited and finalized.
The study was supported by the QUEST Center for Transforming Biomedical Research at the Berlin Institute of Health and by the German Research Foundation (DFG, Deutsche Forschungsgemeinschaft) under Germany´s Excellence Strategy-EXC-2049-390688087. The authors have no conflicts of interest to declare.

Please address all correspondence to:
Pawel Fidzinski
Chariteplatz 1
10117 Berlin, Germany
Email: Pawel.fidzinski@charite.de

We look forward to hearing from you at your earliest convenience.

Yours sincerely,

Larissa Kraus, MSc

TITLE:

Preparation of Acute Human Hippocampal Slices for Electrophysiological Recording

AUTHORS AND AFFILIATIONS:

Larissa Kraus[1], Laura Monni[1,2], Ulf C. Schneider[3], Julia Onken[3], Philipp Spindler[3], Martin Holtkamp[1], Pawel Fidzinski[1,2]

[1]Charité-Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Neurology with Experimental Neurology, Berlin, Germany

[2]Charité-Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, NeuroCure Cluster of Excellence, Berlin, Germany

[3]Charité-Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, of Neurosurgery, Berlin, Germany

Corresponding Author:

Pawel Fidzinski (pawel.fidzinski@charite.de)

Email Addresses of Co-authors:

Larissa Kraus (larissa.kraus@charite.de)
Laura Monni (laura.monni@charite.de)
Ulf C. Schneider (ulf.schneider@charite.de)
Julia Onken (julia.onken@charite.de)
Philipp Spindler (philipp.spindler@charite.de)
Martin Holtkamp (martin.holtkamp@charite.de)

KEYWORDS:

electrophysiology, epilepsy, drug development, human, hippocampus, ex vivo

SUMMARY:

The presented protocol describes the transport and preparation of resected human hippocampal tissue with the ultimate goal to use vital brain slices as a preclinical evaluation tool for potential antiepileptic substances.

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 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[6–11].

In contrast, living resected human brain tissue has been investigated concerning preclinical drug evaluation, basic neuronal functions and gene expression patterns[12–17]. 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 analytic 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[20–22].

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).

1.1. For individual 10x solutions, add substances to ddH2O according to **Table 1** and stir until dissolved.

1.2. Use 10x solutions up to 1 month after preparation (up to 1 year for frozen 10x choline aCSF).

1.3. 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.

1.4. 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 as early 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).

2.1. Choline aCSF for transport and preparation

2.1.1. For the final 500 mL solution, thaw 50 mL of the 10x solution 1.1 aliquot for choline aCSF

in 37 °C water bath.

2.1.2. Add 50 mL of the 10x solution 1.1 aliquot and 50 mL of 10x solution 2 to approximately 300 mL of ddH_2O.

2.1.3. Add final concentrations of glucose and MgCl, then stir until dissolved (**Table 1**, solution 1.1).

2.1.4. Add ddH_2O to a final volume of 500 mL and measure osmolarity (300 mOsm ± 10 mOsm).

2.1.5. Optionally, use a filter to sterilize the solution (see discussion on prolonged slice viability under sterile conditions).

2.1.6. Fill a separate bottle with approximately 100 mL of 1x choline aCSF for transport from the operation room to laboratory.

2.1.7. 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.

2.1.8. Store the final solution at 4–8°C until further use.

2.1.9. 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.

2.2. aCSF for storage and recording

2.2.1. 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.

2.2.2. Add ddH2O to a final volume of 2 L and measure osmolarity (300 mOsm ±10 mOsm).

2.2.3. Prewarm the solution to 35 °C and carbogenate for at least 10–15 min before use.

2.3. HighK^++4-AP aCSF for induction of burst activity

2.3.1. 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.

2.3.2. Add glucose and 4-AP (final concentration = 100 µM) according to **Table 1**.

2.3.3. Add ddH_2O to final volume of 1 L and measure osmolarity (300 mOsm ±10 mOsm).

2.3.4. Prewarm the solution to 35 °C and carbogenate for at least 10–15 min before use.

2.4. lowMg^{2+}+BIC aCSF for induction of SLEs

2.4.1. 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.

2.4.2. Add glucose and BIC (final concentration = 10 µM) according to **Table 1**.

2.4.3. Add ddH_2O to final volume of 1 L and measure osmolarity (300 mOsm ± 10 mOsm).

2.4.4. Prewarm the solution to 35 °C and carbogenate for at least 10–15 min before use.

3. Preparation of interface chamber

3.1. In an interface chamber, rest the slices 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 (here, interface chambers consist of two compartments) and place them on top of each other.

3.2. 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 and thin-cut strings ~10 cm in length are used; for placement, see **Figure 1**).

3.3. 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.

3.4. Ensure an aCSF flow rate of 1.8 mL/min with a peristaltic pump.

3.5. 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.

4.1. Wipe the preparation area with 70% EtOH and place either aluminum foil or sterile covers on top of the area.

4.2. 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.

4.3. 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.

4.4. 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

5.1. 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.

5.2. Remove tissue from choline aCSF and cut away any burned portions of tissue.

5.3. 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.

5.4. Slice the brain tissue into 400 µm thick slices and adjust the amplitude and speed during cutting. Due to connective tissue and 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 thick 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.

5.5. 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).

5.6. 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.

5.7. Slices can be recorded for up to 20 h (even longer when under sterile conditions).

6. Recording of epileptiform activity

6.1. 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.

6.2. 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.

6.3. 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.

6.4. 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.

6.5. Use a heating element connected to the inflow in close proximity to the membrane chamber to ensure a stable temperature of 32 °C.

6.6. Prepare 1–2 MΩ glass pipettes using a vertical puller. Fill pipettes with 154 mM NaCl solution and place them in an electrode holder.

6.7. 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.

6.8. 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.

6.9. Place electrodes in the region and layer of interest (here, CA1) and begin recording.

6.10. Record field potential activity in current clamp mode with a sampling rate of 10–20 kHz and low-pass filtered at 2 kHz.

6.11. Record basal activity in aCSF for up to 5 min.

6.12. 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.

6.13. 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.

6.14. 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.

6.15. In the case that baseline activity is stable (plateau of event frequency), wash in the desired drug. Note that the high flow rate wash in of drugs takes only 2–5 min, allowing for fast solution exchange.

6.16. Record activity during a 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

7.1. 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.

7.2. 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].

7.3. 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 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. This activity was able to be 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 AND TABLE LEGENDS:

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).

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].

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. It then increased 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].

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[17,20–22]. 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 in only one patient has been tested. 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 seizure-like events (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 an 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.

ACKNOWLEDGMENTS:

We thank Mandy Marbler-Pötter (Charite-Unversitätsmedizin, Berlin) for excellent technical assistance. P.F. was funded by the German Research Foundation (DFG, Deutsche Forschungsgemeinschaft) under Germany´s Excellence Strategy-EXC-2049-390688087. This work has been supported by the QUEST Center for Transforming Biomedical Research at the Berlin Institute of Health.

DISCLOSURES:

The authors declare no conflict of interest.

REFERENCES:

1. Hirtz, D. et al. How common are the “common” neurologic disorders? *Neurology*. **68** (5), 326–37 (2007).
2. Ngugi, A.K., Bottomley, C., Kleinschmidt, I., Sander, J.W., Newton, C.R. Estimation of the burden of active and life-time epilepsy: a meta-analytic approach. *Epilepsia*. **51** (5), 883–90 (2010).
3. Kwan, P., Brodie, M. J. Early Identification of Refractory Epilepsy. *New England Journal of Medicine*. **342** (5), 314–319 (2000).
4. Löscher, W. Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. *Seizure*. **20** (5), 359–368 (2011).
5. Ledri, M. *et al.* Differential Effect of Neuropeptides on Excitatory Synaptic Transmission in Human Epileptic Hippocampus. *The Journal of Neuroscience: the official journal of the Society for Neuroscience*. **35** (26), 9622–31 (2015).
6. Qi, X.R. *et al.* Alterations in the steroid biosynthetic pathways in the human prefrontal cortex in mood disorders: A post-mortem study. *Brain Pathology*. **28** (4), 536–547 (2018).
7. Verwer, R. W. H. *et al.* Post-mortem brain tissue cultures from elderly control subjects and patients with a neurodegenerative disease. *Experimental Gerontology*. **38** (1–2), 167–72 (2003).
8. Verwer, R. W. H. *et al.* Cells in human postmortem brain tissue slices remain alive for several weeks in culture. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. **16** (1), 54–60 (2002).
9. Le Maître, T. W., Dhanabalan, G., Bogdanovic, N., Alkass, K., Druid, H. Effects of Alcohol Abuse on Proliferating Cells, Stem/Progenitor Cells, and Immature Neurons in the Adult Human Hippocampus. *Neuropsychopharmacology*. **43** (4), 690–699 (2018).
10. Dennis, C. V., Suh, L. S., Rodriguez, M. L., Kril, J. J., Sutherland, G. T. Human adult neurogenesis across the ages: An immunohistochemical study. *Neuropathology and Applied Neurobiology*. **42** (7), 621–638 (2016).

11. Verwer, R. W. H. *et al.* Mature astrocytes in the adult human neocortex express the early neuronal marker doublecortin. *Brain*. **130** (12), 3321–3335 (2007).
12. Ting, J. T. *et al.* A robust ex vivo experimental platform for molecular-genetic dissection of adult human neocortical cell types and circuits. *Scientific Reports*. **8** (1), 8407 (2018).
13. Le Duigou, C. *et al.* Imaging pathological activities of human brain tissue in organotypic culture. *Journal of Neuroscience Methods*. **298**, 33–44 (2018).
14. Agostinho, A. S. *et al.* Dynorphin-based "release on demand" gene therapy for drug-resistant temporal lobe epilepsy. *EMBO molecular medicine*. **11** (10), e9963 (2019).
15. Hodge, R. D. *et al.* Conserved cell types with divergent features in human versus mouse cortex. *Nature*. **573** (7772), 61–68 (2019).
16. Beaulieu-Laroche, L. *et al.* Enhanced Dendritic Compartmentalization in Human Cortical Neurons. *Cell*. **175** (3), 643-651.e14 (2018).
17. Sandow, N. *et al.* Drug resistance in cortical and hippocampal slices from resected tissue of epilepsy patients: no significant impact of p-glycoprotein and multidrug resistance-associated proteins. *Frontiers in Neurology*. **6**, 30 (2015).
18. Wickham, J. *et al.* Prolonged life of human acute hippocampal slices from temporal lobe epilepsy surgery. *Scientific Reports*. **8** (1), (2018).
19. Avoli, M., Jefferys, J. G. R. Models of drug-induced epileptiform synchronization in vitro. *Journal of Neuroscience Methods*. (2015).
20. Kraus, L. *et al.* Dimethylethanolamine Decreases Epileptiform Activity in Acute Human Hippocampal Slices in vitro. *Frontiers in Molecular Neuroscience*. **12**, 209 (2019).
21. Antonio, L. L. *et al.* In vitro seizure like events and changes in ionic concentration. *Journal of Neuroscience Methods*. (2016).
22. Gabriel, S. *et al.* Stimulus and Potassium-Induced Epileptiform Activity in the Human Dentate Gyrus from Patients with and without Hippocampal Sclerosis. *Journal of Neuroscience*. **24** (46), 10416–10430 (2004).
23. Haas, H. L., Schaerer, B., Vosmansky, M. A simple perfusion chamber for the study of nervous tissue slices in vitro. *Journal of Neuroscience Methods*. **1** (4), 323–325 (1979).
24. Hill, M. R. H., Greenfield, S. A. The membrane chamber: a new type of in vitro recording chamber. *Journal of neuroscience methods*. **195** (1), 15–23 (2011).
25. Andersson, M. *et al.* Optogenetic control of human neurons in organotypic brain cultures. *Scientific Reports*. **6**, 24818 (2016).
26. Ting, J. T., Daigle, T. L., Chen, Q., Feng, G. Acute brain slice methods for adult and aging animals: application of targeted patch clamp analysis and optogenetics. *Methods in molecular biology (Clifton, N.J.)*. **1183**, 221–42 (2014).
27. Testa-Silva, G. *et al.* Human synapses show a wide temporal window for spike-timing-dependent plasticity. *Frontiers in Synaptic Neuroscience*. **2**, 12 (2010).
28. Ting, J. T. *et al.* Preparation of Acute Brain Slices Using an Optimized N-Methyl-D-glucamine Protective Recovery Method. *Journal of Visualized Experiments*. (132), (2018).
29. Morris, G., Jiruska, P., Jefferys, J. G. R., Powell, A. D. A New Approach of Modified Submerged Patch Clamp Recording Reveals Interneuronal Dynamics during Epileptiform Oscillations. *Frontiers in Neuroscience*. **10**, 519, (2016).
30. Valeeva, G., Valiullina, F., Khazipov, R. Excitatory actions of GABA in the intact neonatal rodent hippocampus in vitro. *Frontiers in Cellular Neuroscience*. (FEB), doi:

615 10.3389/fncel.2013.00020 (2013).
616

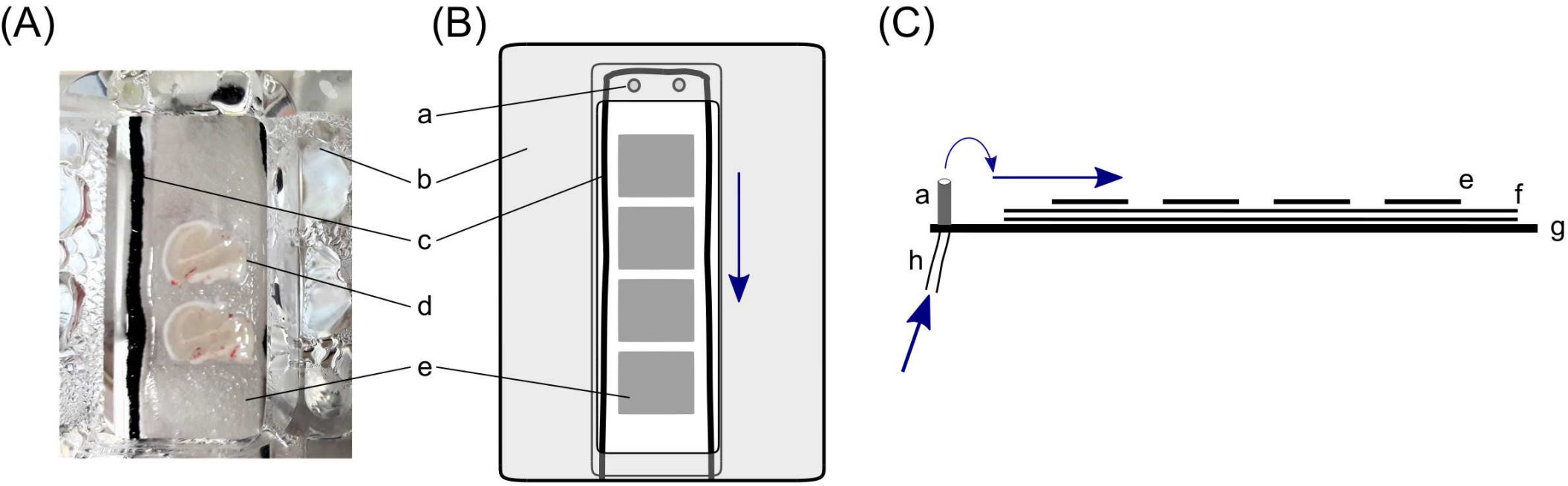

Figure 2

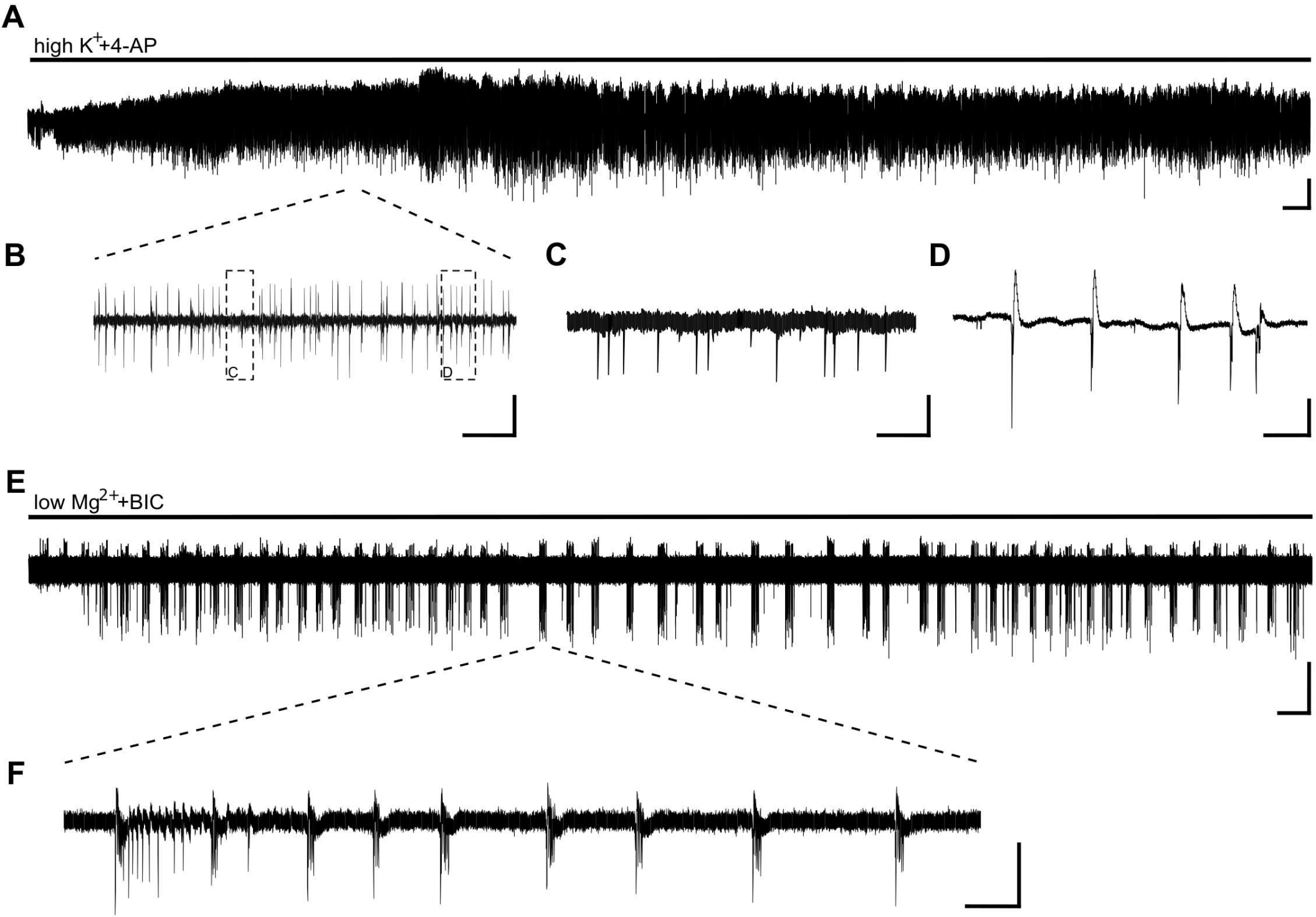

Figure 3

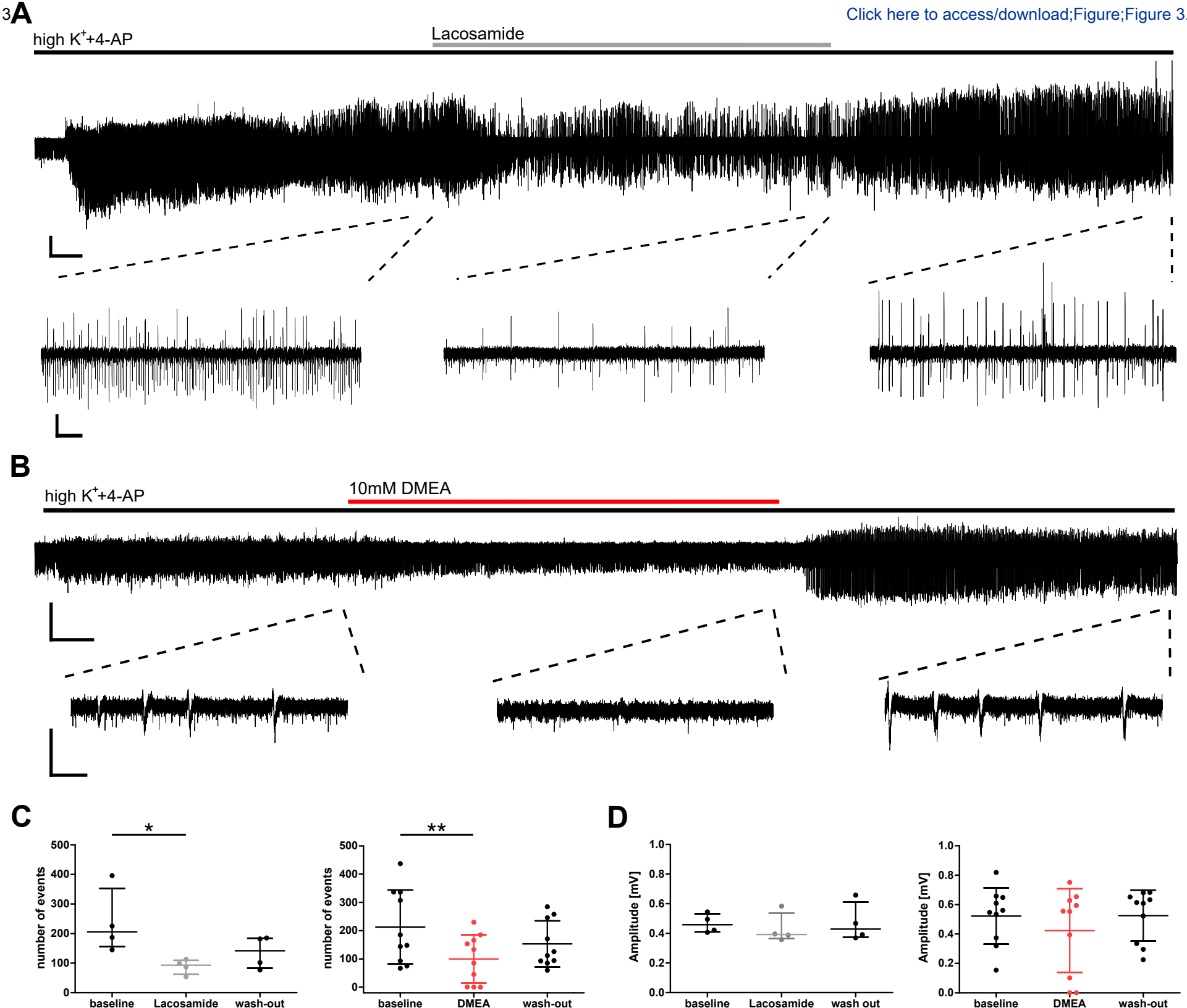

Table 1 Click here to access/download;Table;Table 1.xlsx

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 lowMg^{2+}+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	

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
(+)-Na L-ascorbate	Sigma Aldrich	A4034	
4-AP	Sigma Aldrich	275875-5G	
Blades	eliteSERVE GmbH	HW3	used for the vibratome
$CaCl_2$	Merck	102382	
Choline Cl	Sigma Aldrich	C1879	
Filter paper	Tiffen	EK1546027T	
Gas-tight bottle caps	Carl Roth GmbH+Co.KG	E694.1	
Glass filaments	Science Products	GB150F-8P	for recording electrodes
Glass gas disperser	DWK Life Sciences GmbH	258573309	
Glucose	Sigma Aldrich	G7528	
Interface Chamber	inhouse made	-	see Haas et al., 1979
KCl	AppliChem	131494.1210	
Membrane (Cell culture inserts)	Merck	PICM030050	
Membrane chamber	inhouse made	-	see Hill and Greenfield, 2011
$MgCl_2 \cdot 6H_2O$	Carl Roth	HNO3.2	
$MgSO_4$	Sigma Aldrich	M7506	
Na pyruvate	Sigma Aldrich	P8574	
NaCl	Carl Roth	3957.1	
NaH_2PO_4	Merck	106346	
$NaHCO_3$	Carl Roth	HNO1.2	
Peristaltic pump	Gilson	Minipuls 3	
Slice holder	Warner instruments	SHD-41/15	
Vertical puller	Narishige	PC-10	
Vibratome	Leica	VT1200S	

Editorial comments:

General:
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
2. Please use 'L' instead of 'l' to represent liters (including 'mL').
3. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please limit the use of commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.
For example: Falcon

Answer: According to your suggestions, we have proofread and re-edited the manuscript where necessary. All abbreviations referring to "liters" have been changed to 'L' and commercial language has been removed from the manuscript.

Protocol:
1. Pictures and/or diagrams of the chamber(s) used here may be useful.

Answer: We agree and have added pictures of the interface chamber (see Figure 1). We also added a reference including pictures for the membrane chamber used for recordings.

2. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. If revisions cause a step to have more than 2-3 actions and 4 sentences per step, please split into separate steps or substeps.
Specific Protocol steps:
1. 2.1.7, etc.: How exactly do you carbogenate?
2. 3.1, 3.3: How large, exactly, are the pieces of filter paper? Where are they placed?
3. 3.2: It is unclear how exactly the cotton strings are used.
4. 5.5: How do you 'reduce size', and how large should slices be?
5. 6.1: What membrane is being discussed her? It is not in the Table of Materials nor has it been discussed previously. Where exactly does it go?
6. 6.2: How are pipettes prepared?
7. 6.7, etc.: How exactly are solutions washed in?

Answer: Thank you for these comments, we have adapted all sections of the protocol according to the given suggestions.

Figures:
1. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account.

Answer: The results and figures presented in this manuscript are reused from our previous publication (Kraus et al., 2019) published with Frontiers. Under the Frontiers Terms and Conditions, authors retain the copyright to their work. All Frontiers articles are Open Access and currently distributed under the terms of the Creative Commons Attribution License (CC-BY, version 4.0), which permits the use, distribution and reproduction of material from published articles, provided the original authors and source are credited. You can access Frontiers copyright statement here: https://www.frontiersin.org/legal/copyright-statement.

References:
1. Please do not abbreviate journal titles.
Answer: We have changed our citation style to fit your comment.

Table of Materials:
1. Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.

Answer: According to your suggestions, we have proofread and now mention all materials and equipment.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

Kraus et al. describe the preparation of human hippocampal slices as an experimental tool to investigate the efficiency of AED on induced epileptiform activity in the hippocampus. Using human tissue samples as a translational tool has been used for decades and are widely used to study physiology and pathology of human circuits. The idea of this article is to standardize the induction of seizure like activity is interesting and might proof beneficial to compare data sets of different studies.

Answer: We thank the reviewer for his positive assessment of our manuscript.

The authors suggest to use high k+ 4AP as a preclinical test of new anticonvulsive compounds, since the authors found this to induce the most robust seizure like activity (SLA). This might be in principal a useful idea, the limitations should nevertheless be clearly discussed and some of the parameters are a little random (see in specific comments).

Answer: We address this issue as detailed below.

I clearly miss a picture/staining of a slice that is a quality control to see what of a network is producing the activity (see specific point 1). It might be possible that a drug is more effective on certain cells and even areas of the human brain and might therefore be not effective in the measured model, but still potent in a patient. Such a limitation should be considered in the discussion. Maybe additional measurements in human Cortex might be needed if a drug is not working in the Hippocampus?

Answer: We address this issue as detailed below.

Minor Concerns:

Specific comments to improve the manuscript:

1) Add a representative picture of a slice that would be considered minimum network that can be used for the described approach to test a drug. Or a range of slices that go from best - to still good enough for this model. Do you need CA1 and DG at minimum? Would it be of interest to record from several areas?

Answer: Thank you for this comment. Determining the recorded or the minimum network necessary for the presented recordings is not a straightforward task as the size of slice or the number of visualized somata not necessarily correlates with network size. Network size in slices is dependent on various factors, including not only the size of the slice but also slice thickness, the slicing angle with respect to the surface and finally the specific region the slice was obtained from.

In our work, we use 400 µm thick slices and we aim to use slices as large as possible (limited only by the size of the recording chamber and the size of tissue block received). In addition, in patients with temporal lobe epilepsy and varying degrees of hippocampal sclerosis (HS) we expect also a large variety of neuronal survival, which likewise impacts the network size. However, we have not observed differences in induction of epileptiform activity between low or high neuronal cell loss due to HS (as assessed by Wyler classification, see suppl. Table 1, Kraus et al., 2019).

Hippocampal slices used in our work include CA1, CA2 and CA3 regions and, if preserved, the dentate gyrus. In these slices we were able to robustly induce seizure like activity, therefore we assume that a CA1-CA3 network is large enough for drug testing.
We agree that preclinical evaluation of a drug is limited by the availability of its target. If the drug target is known, analysis concerning expression and localization can be performed prior to electrophysiological recordings to determine area of interest in the hippocampus (e.g. CA1 vs DG) or even cortex.
Concerning CA1 vs DG, we have seen most reliable induction of epileptiform activity in CA1, simply because it is in most cases intact after resection and despite high neuronal cell loss in CA1 region of hippocampus with HS. DG as well as subiculum are frequently cut or damaged during resection, which might impact induction of epileptiform activity.

2) Parameters: Why "Slice brain tissue to 400 µm thick"? Did the authors test any different thickness? and "Before collecting, reduce size of brain slices to fit into recording chamber", what is essential to keep for the model to work? See also point 1.

Answer: Thank you for this comment, we agree that slice thickness should be discussed and have edited our manuscript (see 266-272). Concerning what is essential to keep for the model: As mentioned in the answer of the previous question, we have also recorded epileptiform activity in hippocampal brain slices where connection between DG and CA3 was cut and subiculum was missing. This did not affect our induction or recording in CA1.

3) Clearly the limitations of the design should be stated (variability of data set, drug history of patients, etc.). Especially, the drug treatment history and potential refractory towards a drug seems interesting. Did the authors test any patient that was refractory to Lacosamide (or any other drug if information was available) in vitro? This would be quite nice, if such correlations could be established and studied with this model.

Answer: We agree with the comment and discussed the limitations such as clinical data variability more thoroughly (see line 497-508). Certainly, possible correlations between effects in vitro and previous history are highly interesting. However, the relative low patient numbers present an important limitation for detection of such correlations and have so far not been investigated.
Regarding aspects of pharmacoresistance, we do not expect to reproduce clinical features of pharmacoresistance in our in vitro model. Although we deal with human tissue, the neuronal network within a slice is artificial (e.g. needs induction in order to produce seizure like activity) and cannot be compared to an in vivo situation.

4) "In slices obtained from one patient, low Mg2+ and BIC were applied and showed induction of SLEs in CA1 area. However, induction of SLEs in resected human hippocampus is not as robust as induction of burst activity. Slices often switch from SLEs to burst activity or do not show induction of SLEs at all." This is an odd statement. It is not easy to draw a conclusion from one patient and other studies used exactly such induction (Low magnesium) to induce stable epileptiform activity (Straub et al. 2001). Please clarify.

Answer: We agree that the above mentioned statement is confusing and have edited the manuscript (see line 442-449).

5) How many measurements per patient are suggested, since the authors average them to quantify the effect.

Answer: The number of measurements per patient are determined statistically and depend on the effect size as well as the statistical power. Due to statistical quality considerations, we define one patient as one sample, meaning we cannot compare recordings between slices of the same patient. Therefore, when we perform the same measurements in 2 or more slices of the same patient we average for the final statistical analysis. Repeating the same measurements in one patient will therefore not increase the sample size but can increase the number of possible substances to be tested. With regard to slice availability, a limited amount of slices is available from a hippocampal resection and we always consider to include a positive (e.g. lacosamide) and a negative control (no drug application) and try to avoid repeating measurements in the same patient more than twice.

6) Terminology : interictal spikes cannot occur in a slice.

Answer: Thank you for this comment; we have edited out manuscript (see legend of Fig. 2, line 414).

Reviewer #2:

Manuscript Summary:

The protocol described here is indeed very interesting and could help researchers to replicate a fundamental technique of electrophysiological study of epileptic human tissue. Hippocampal slices are a technically difficult preparation and a step by step guide could enhance the replicability of this method. Overall, the paper addresses many key aspects of the matter, since also the important issues of tissue transportation and handling are sufficiently well-treated. Furthermore, the representative results provide a useful example of epileptiform activity induced by the protocols described in the paper. Some examples of how two widely used AEDs affect the epileptic burst activity are shown, and give a nice insight on the pharmacological implications of the method.

Answer: We thank the reviewer for the positive comments.

There are some details that could be better elucidated to make the protocol more immediately comprehensible:

Major Concerns:

DISCUSSION:

-Lines 375-380: The human slice preparation is indeed a very useful technique but, nonetheless, like all experimental procedures, it comes with limitations. These latter aspect is not sufficiently addressed in the paper and should be at least mentioned for the sake of the reader.

For example, there is extensive literature addressing the issue of "slicing-induced neuronal damage" (see Valeeva et al., 2013; Front Cell Neurosci) which is a technical downside that cannot be avoided while trying to reproduce this technique and not only a consequence of technical errors (wrong slicing angles or long transportation times).

Answer: Thank you for this comment. We agree that the limitations of the technique should be discussed and edited our discussion accordingly (see line 497-502).

-Brain slices are not the only possible way to record electrophysiological responses from "human channels and/or receptors". It is indeed true that this model is very near to the complexity of human diseases and network but nonetheless other techniques can't be ignored and need to be taken into account and at least briefly mentioned here. Recently, significant advancements have been done with techniques such as iPSC (see Livesey et al., 2012; Tang et al., 2014, 2016) or membrane microtransplantation (Ruffolo et al., 2016, 2018) which enable electrophysiological studies of patients from whom it is normally difficult to obtain viable brain tissue.

Answer: Thank you for this comment, however we do not entirely agree with this view. iPSCs and membrane microtransplantations are valuable tools to investigate channel and receptor functions. Here our main goal was to establish a method to validate drug efficacy in a neuronal network, which represents an adult diseased brain. Even iPSCs, which form a network will only ever represent developing embryonal neuronal networks and therefore will remain important in studying complex cell-to-cell interactions in detail but be limited as a drug evaluation tool. Human brain tissue *ex vivo* certainly has limitations (see discussion line 502-508) and should be seen as an additional preclinical evaluation tool, though in our opinion it has features which cannot be reproduced by culture systems.

Minor Concerns:
PROTOCOL:
-Line 107: quantify "longer"

Answer: see line 111

-Solutions: specify the exact pH of the solutions

Answer: see line 126

-Even if how to analyze "SLEs" is described elsewhere, it would be recommendable to add at least a brief description of the electrophysiological parameters that define such events, for rapid reference while reading the method. Same applies for burst activity: how do you define it? These aspects may seem trivial but could facilitate a broader comprehension of the text.

Answer: Thank you for this comment. We agree that even though the analysis of the induced activity is not the main focus of the manuscript, we should extend the descriptions. We edited our manuscript accordingly (see line 353-373).

FIGURES:
Even if the results here presented are only representative figure legends could nonetheless include some additional details. It is not clear, for example, at which concentration were the drugs used (Lacosamide and DMEA), same for the concentration of other mentioned chemicals (highK+, 4-AP. Concentration?)

Answer: We agree and thank you for this comment. We have edited our figure legends.

REPRESENTATIVE RESULTS
-Line 304-306: Please specify "TLE" patients.

Answer: See line 384

-Lines 309-320: A brief description of highK++4-AP vs lowMg2++BIC is done here. The Authors conclude that for "preclinical evaluation of entiepileptic compounds burst activity in CA1 is more reliable and stable than lowMg2++BIC. This sentence is not entirely clear. Did the Authors mean that the evaluation of burst activity in CA1 after application of highk+/4AP is more reliable? Please rephrase this sentence in order to make it easier to understand.

Answer: We agree that this statement is misleading and have edited this in the results and discussion of our manuscript (see line 393-394 and line 442-449).

Furthermore, can the authors add some references to support the statement at lines 318-320?
Answer: After careful consideration, we edited this part and removed the above-mentioned sentence.

Reviewer #3:

Manuscript Summary:

A method for preparing, maintaining, and using acute slices from human surgical brain tissue. Extracellular electrical recording of intrinsic dynamics and driven epileptiform activity, as well as modulation by exogenous compounds, is provided.

Major Concerns:

None

Minor Concerns:

The authors may want to discuss other methods of preparing slices that do not involve Choline, which is a controversial reagent in the acute slice field, even in rodents. The figures of the electrophysiology figures are not intuitive and could use some design/visualization improvement.

Answer: Thank you for this comment, however, we included a discussion on the use of NMDG as alternative solution for preparing slices (see line 463-473).