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Easy to build cost-effective acute brain slice incubation system for parallel analysis of multiple treatment conditions

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ABSTRACT

Background: Acute brain slices represent a powerful tool for analysis of brain function in physiology and pathology. Commercial systems and custom-build solutions with carbogen (95% O_2/5% CO_2) aeration, but they are expensive, have a high working volume requiring large amount of substances, and only limited options for treatment in parallel are possible.

New method: We developed a novel cost-effective incubation system using materials available in every laboratory, allowing parallel incubation of several treatment conditions, thus also reducing the number of experimental animals. Our system incubation parameters were optimized for cortical neuron observation.

Results: We tested several different options using 6, 12 or 24 standard culture well plates, combining them with cell strainer baskets inside. The system was placed in a pre-warmed incubator at 37 °C. Carbogen was injected through a 22 gauge needle, positioned between the basket and the wall of the well. Best results were achieved in a 6-well plate. In 12 and 24-well plates bubbles accumulated beneath the basket, displacing it upwards, making it unsuitable for our purposes. The gas oxygenized the medium without mechanically disturbing the slices, protected within the strainer basket, but still allowing optimal diffusion through the 100 μm pores. In a 6-well plate, six simultaneous treatments were possible in parallel. LDH/Cytotoxicity tests showed an acute toxicity of less than 7%. The system lost about 2.5% per hour of the fluid through evaporation, which was replenished every 2 h. Up to 6 h after treatment, however, this evaporation was excellently tolerated by the neurons even without fluid replenishment, most probably due to the anti-swelling effect of the mildly hypertonic medium. We performed two staining procedures, working excellently with this experimental setup, namely – a modified DiI staining and a slice silver impregnation method, both confirming the intact neuronal morphology. Preserved CA3 calcium influx and removal response following KCl depolarization confirmed the normal physiology of the pyramidal neurons 6 h after exposure in the system.

Comparison to existing methods: The proposed system is much cheaper than the commercial solutions, can be constructed in any lab, allows up to 6 different treatments in parallel, which none of the existing systems allows. Antibiotic presence in the incubation medium and adequate evaporation control is required if longer incubation (> 6 h) is needed. Lower incubation volumes (3–6 ml) allow sparing expensive reagents. Our procedure was optimized for cortical neurons, further fine tuning to meet other specific requirements is possible.

Conclusions: The system we propose allows filling the gap for budget solutions for short to mid-term incubation of acute brain slices.

Abbreviations: aCSF, artificial cerebrospinal fluid; LDH, lactate dehydrogenase; NMDG, N-methyl-D-glucamine; DiI, 1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyaninperchlorat.

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1. Introduction

The acute brain slice experimental system has been established more than half a century ago in a milestone work by Li and McIlwain (1957), utilizing brain tissues of adolescent and adult animals. The technical performance and setup of Li and McIlwain, as well as the electrophysiological recordings represent a masterwork of the experimental neurosciences. In the initial experimental setup, the analyzes of slices were performed within short periods of time (15–30 min), continuously extending them to hours. As an alternative of the acute slices, allowing for better imaging and electrophysiological recordings, organotypic slice cultures can be prepared on membranes for days and weeks at the interface between air and medium (Stoppini et al., 1991; Lossi et al., 2009). The drawbacks of the organotypic cultures versus acute slices are the significant reorganization of neuronal circuitry (Lossi et al., 2009), and the subsequent massive glial reaction (Staal et al., 2011). They are usually done with younger tissues (up to postnatal day 4), however multiple protocols for ex vivo organotypic culturing of the adult brain exist (Angeles et al., 2018; Tan et al., 2018; Kim et al., 2013; Wilhelmi et al., 2002). Maturation stage of human brain at birth corresponds to postnatal day 6–7 in rodents (Semple et al., 2013), therefore slices from animals beyond day 7 after birth are best suited for studying postnatal physiological and pathological effects.

Analysis of various types of acute brain slices (mostly hippocampal and cerebellum, but also cortical) allowed defining several obligatory key parameters – oxygenation by carbogen (a mix of 95% O_2/5% CO_2) in artificial cerebrospinal fluid (aCSF, carbonate-buffered solution), supplemented with glucose. Further critical elements of incubation are temperature, pH and diminishing post-sectional swelling. Temperature control remains purely technical and pH control is largely achieved by the nature of the buffer system. Against swelling, different experimental adaptive approaches such as cutting at 4 °C, at 37 °C, using sucrose during cutting as an anti-swelling tool (Aghajanian and Rasmussen, 1989), glycerol instead of NaCl (Ye et al., 2006) or incubation in NMDG-containing medium as sodium substitute are used (Ting et al., 2014).

Technically, various incubation setups exist, many are commercial, some are custom-built. In some experiments, slices are stored in a carbogenated storage chamber such as the Brain Slice Keeper-4 (AutoMate Scientific) or similar, using one slice at a time for imaging in a perfusion chamber on an electrophysiological or microscopy setup (Larkman et al., 1988). Longer-term incubations at physiological temperatures are performed either in medium/buffer supplemented with antibiotics or in special systems, maintaining constant pH and antibacterial numbers by UV irradiation (e.g. the Braincubator) (Buskila et al., 2014). A disadvantage of such systems is the lack of multiple parallel incubation capabilities, as well as their price.

The aim of our current work is to demonstrate a very easy and cost-effective system we have consecutively developed and successfully used for years (Wippel et al., 2011, 2013; Hupp et al., 2019).

2. Materials and methods

2.1. Animals and brain slice preparation

Acute slices were prepared from the brains of newborn C57Bl/6JRj mice at postnatal day (PD) 10–12. After opening of the skull and removal of the cranial plates, the brain was transferred to PBS. The cerebellum was cut off and discarded, the remaining cerebrum was glued cut-face down to a specimen holder with Vetbond (3M Company, Saint Paul, Minnesota, USA). 340 µm thick sections were prepared on a Leica VT1000 S sectioning vibratome (Leica Microsystems GmbH, Wetzlar, Germany). Coronal sectioning was carried out starting around 1 mm rostral from the bottom of the olfactory bulb, up to around 4 mm down, at the level of the sensomotor cortex. During preparation, the brains were kept in constantly oxygenated (95% O_2/5% CO_2 mix, Carbagas Depot F. + H. Engel, Bern, Switzerland) artificial cerebrospinal fluid (aCSF) at room temperature, containing glucose (12.5 mM) and 1% penicillin/streptomycin (all from Gibco, Thermo Fisher Scientific (Switzerland) AG, Basel, Switzerland). For short time storage, the slices were kept in oxygenated aCSF at room temperature in cell strainers (100 µm pore size, Corning, Inc., Corning, New York, USA). For recovery, the slices in cell strainers were kept for 30 min in a standard non-CO_2 incubator at 33 °C in aCSF in a 6-well plate (under constant oxygenation), before increasing the temperature of the incubator to 37 °C for a subsequent treatment period of 5–12 h (also under constant oxygenation). Evaporation of medium during incubation was substituted with 330 µl sterile water every two hours.

ACSF was prepared using the following components (all from Carl Roth GmbH + Co. KG, Karlsruhe, Germany):

	Molarity (mmol/l)
NaCl	119 mM
KCl	2.5 mM
NaH_2PO_4	1.2 mM
$NaHCO_3$	25 mM
Glucose	12.5 mM
$MgSO_4$	2 mM
$CaCl_2$	2 mM

1% penicillin/streptomycin (Thermo Fisher) was added and the pH adjusted to 7.3–7.4. This yielded a solution with an osmolarity of 300–310 mOsm, which was subsequently filtered through a 0.22 µm pore sterilising filter and pre-warmed to 33 °C or 37 °C according to the protocol step.

2.2. Long-term incubation chamber

A detailed construction protocol can be found in Supplementary file 1. Conceptual diagram of the system is shown in Fig. 1. In Fig. 2, the exact preparation of the cell strainer, which serves as an incubation basket, is presented. For stability of the inserted needle, a double cover system on the top of the 6-well plate is proposed. In this way, both covers can be removed away together without displacement of the needle or problems with repositioning it later on next to the basket. The needle opening in the plastic plate cover was made using a flame-heated needle. On the top of both covers, a hole with a diameter of 4–5 mm was drilled, allowing adding fluids/substances into the wells. The holes are covered between treatments to minimize evaporation.

2.3. LDH/cytotoxicity assay

To analyze the vitality of the slices in the culture, a standard LDH release assay (Promega GmbH, Mannheim, Germany) for cell damage was performed according to the manufacturer's instructions. Shortly, samples from the aCSF were collected and centrifuged at 5000 rpm

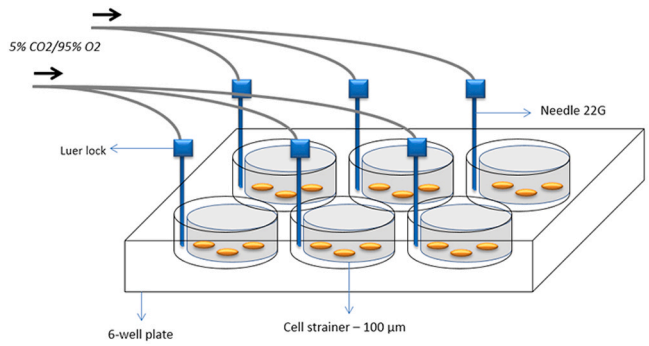

Fig. 1. Schematic diagram of the general configuration of the 6-well plate system with 22 gauge needles and tubing.

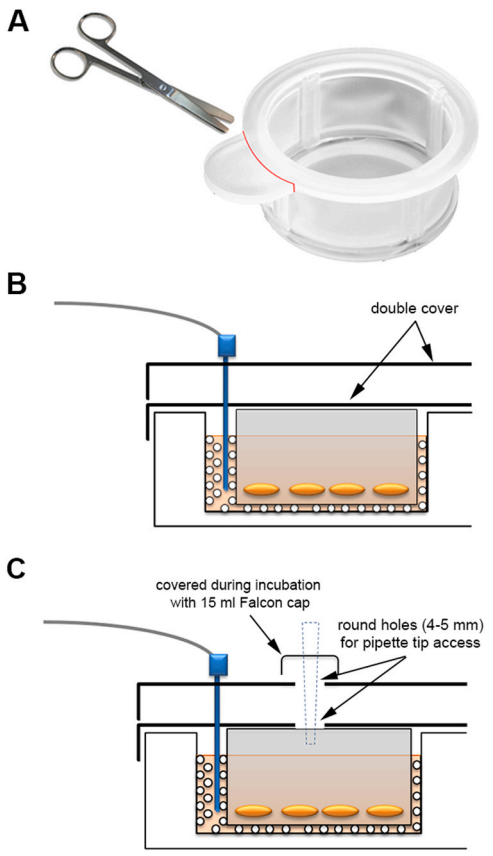

Fig. 2. A. Preparation of the cell strainer for 6-well plate slice incubation by cutting the side knob with scissors. B. Stabilization of the needle position by two plate covers, placed one above the other (they need to be from the same brand to fit perfectly), allowing two-point of needle stabilization and alignment. C. Drilling a 4–5 mm in the middle of both covers allows easy access to the well without lifting the covers. Covering the opening between incubations is needed (e.g. with the cap of a 15 ml conical centrifuge tube) to avoid excessive evaporation.

(better × *g*) for 5 min to remove all cells. 100% lysis control was prepared after parallel incubation of equivalent number of slices with a standardized lysis buffer (Promega) for 4 h, combined with supernatant clearing through centrifugation. The samples were analyzed by a BioTek 800 multiplate absorbance reader (BioTek Instruments, Inc., Winooski, VT, USA).

2.4. Staining of neurons

Following treatment, the brain slices were fixed with 1.5% formaldehyde in aCSF at 4 °C overnight. After washing with 1 × PBS for three times, the slices were placed on a drop of PBS on a piece of Parafilm and excess PBS was removed with a thin transfer pipette. Subsequently, the slices were stained with NeuroTrace™ DiI stain (ThermoFisher) by direct application of the dye onto the cortical region of the slices with a very fine Tungsten needle (Fine Science Tools, North Vancouver, Canada). 5 µl of a 2% solution of DiI crystals in ethanol were dropped on a piece of Parafilm and the Tungsten needle was carefully dipped in it, allowing crystals to precipitate directly on its surface. The DiI crystals on the needle tip are thus much smaller than the ones of the commercially available substance, and the amount of substance applied can be controlled much more precisely. Very low amounts of stain secure much clearer results. Alternatively, DiI tissue labeling paste can be used with the same procedure. The cortices of the slices were carefully punched at the levels of layers III/VI and V. For proper lateral diffusion of the dye along the membranes of the cortical neurons, the slices were kept between two layers of Parafilm (Sigma-Aldrich) in a humidified environment (a drop of PBS was applied onto the slice) for 24 h in the dark at room temperature. After post-fixation of the slices in 4% formalin in PBS for 30 min at room temperature, the slices were washed three times with 1 × PBS and subsequently mounted on glass slides with Mowiol (Sigma-Aldrich). These steps were largely based on the original work of Trivino-Paredes et al. (2019) and modified to allow for placement of lower amounts of DiI.

To visualize the general condition of the tissues, we used another staining approach as well – the brain slice modified Golgi silver impregnation using the SliceGolgi Kit (Bioenno Tech LLC, Santa Ana, CA, USA) according to manufacturer's instructions with slight modifications of the incubation durations. The impregnated slides were mounted on gelatin-coated slides, air-dried, cleared in Xylene and mounted with Entellan (Carl Roth, Karlsruhe, Germany).

2.5. Microscopy and image analysis

The fluorescence images were acquired on a Zeiss LSM 880 using 63x oil immersion objectives (Carl Zeiss AG, Oberkochen, Germany), using either green (488 nm) or red excitation wavelengths (561 nm). Silver stained slices were imaged on a Zeiss Axioimager M2 widefield microscope using either a 40 × dry or a 63 × oil immersion objective (Carl Zeiss AG, Oberkochen, Germany). Image analysis was performed using ImageJ (ver. 1.52p; NIH, Bethesda, USA) with MBF "ImageJ for Microscopy" Collection from Tony Collins and ImageJ (with Fiji add-on package, Johannes Schindelin and team).

2.6. Live imaging of brain slices

Acute brain slices, incubated for 6 h in our experimental system, were stained for 1 h inside the system with Cal520 AM (AAT Bioquest, Sunnyvale, CA, USA) calcium-sensitive dye at final concentration of 5 µM in aCSF. After washing, the slices were rapidly transferred to a custom-built live imaging chamber with coverslip bottom, where they were perfused with carbogenated aCSF at 37 °C using a peristaltic pump. The slices were imaged with 20 × dry objective allowing initial recording of a stable background signal, followed by the addition of KCl in the chamber at a final concentration of 25 mM, starting the washout immediately after the exposure to KCl. Cal520 was imaged once every second using a standard GFP fluorescent filter and minimal fluorescent intensity of 11% on the Cell^M imaging Olympus system. The fluorescent exposure was adjusted to a pulse pattern to minimize unnecessary cytotoxicity outside the window of signal acquisition.

2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism 9.1.0 for Windows (GraphPad Software Inc., La Jolla, CA, USA). For comparison of two groups differing in one parameter, the Mann-Whitney test was applied. p-values below 0.05 were considered statistically significant. The results show the mean value and the standard error of the mean.

3. Results

The initial idea behind our approach was to use cell strainers as immobilization baskets to incubate the slices. If not in baskets, the slices were rapidly destroyed by the fluid movement. Our basket choice fell on classical cell strainers with soft walls and a pore size of 100 µm. Such a system works well in the Brain slice keeper for slice maintenance before incubation, but due to its large volume, it was not applicable for continuous treatments. We tested various incubation combinations to reduce the volume of incubation, but maintain proper oxygenation. A major problem was the accumulation of gas under the basket (custom-made basket in 12-well plate and PluriStrainer in 24-well plate), which subsequently pushed the basket up and out of the fluid (Supplementary

Fig. 1A, B). The solution was the use of 50 ml centrifuge tube cell strainer in a classical 6-well plate (Supplementary Fig. 1C). Here, the gas and the bubbles distributed evenly between the walls of the well and the strainer, and did not accumulate below the strainer. The schematic diagram of the system in a 6-well-plate format is presented in Fig. 1.

The analysis of the setup allowed us to determine several setup optimization steps – removal of the knob of the cell strainer (Fig. 2A), using standard beveled 22 gauge needle, adding a second cover for stabilization and alignment of the needle (Fig. 2B), and drilling an additional hole in both covers to allow easy application of substances without complete removal of the cover (Fig. 2C). The plate was maintained in a normal non-CO_2-incubator, set at 37 °C. The precise amount of gas used for aCSF oxygenation was controlled using a standard Keck tubing clamp.

In the presence of antibiotics (1% penicillin/streptomycin), we failed to detect the growth of any microorganisms. In a series of experiments without antibiotics, following aseptic slice preparation and incubation in sterile-filtered medium and positioning in a normal non-sterile non-CO_2-incubator for 6–8 h, we did not observe the growth of any bacteria either.

Next, we analyzed the lysis of the tissue in the incubation system, using a control incubation system with slices that were aerated on an orbital shaker in a CO_2-incubator. Our carbogen-based incubation system demonstrated approx. 7% LDH release after 8 h of incubation versus > 30% on an orbital shaker (Fig. 3A). Fixation and DiI staining demonstrated adequate configuration of the pyramidal neurons in layers III and V of the neocortex, further confirming the physiological stability of the system (Fig. 3B–D).

Imaging of DiI and silver impregnation was a snapshot of the general condition of the neurons, but was just an indirect marker of physiological intactness of cells. To confirm that the physiological properties were preserved, we incubated slices for 6 h in our incubation system and transferred them to a live imaging perfusion chamber after stained them with the Cal520 calcium-sensitive dye (Fig. 4A). After placement on the microscope, the basal fluorescent signal in the CA3 pyramidal neurons was recorded. Exposure to 25 mM potassium chloride, which induces rapid depolarization and calcium influx in these neurons (Amano et al., 2001) was added and the pattern was followed over several minutes (Fig. 4BFig. 5B, Supplementary movie M1). Initially, calcium influx increased following the potassium-mediated depolarization (preserved membrane potential maintenance), followed by physiologically shaped recovery curves (preserved ability to regulate calcium after the end of stimulation (Ting et al., 2014)).

Supplementary material related to this article can be found online at doi:10.1016/j.jneumeth.2021.109405.

The volume of aCSF we used in the beginning of the incubation was 6 ml. In such conditions, the evaporation loss was approx. 150 µl per hour. Until 6 h post incubation, the slices and the cells tolerated such loss very well, maintaining proper morphology. We used an approach for fluid reconstitution every 2 h, which worked excellently.

During our tests, we used various known modifications for improved incubation such as cutting on ice, maintaining the slices in BME, using sucrose solution for slicing, as well as NMDG-containing medium. In our hands, none of these approaches provided better results than aCSF as judged by morphological dendrite and spine analysis (not shown). Only replacement of the ice incubation with cutting at room temperature allowed to elevate the number of intact spines with 30%, as established by evaluation of confocal images of DiI stained neurons. An overview of

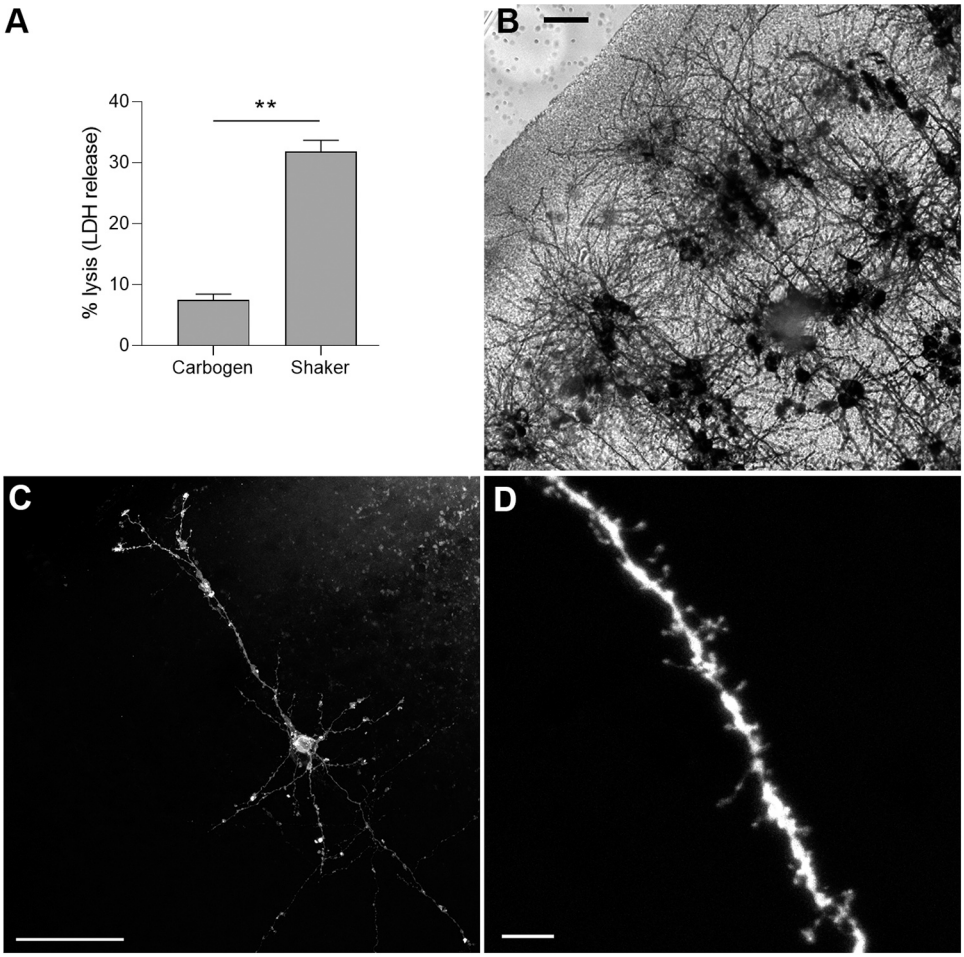

Fig. 3. A. LDH release analysis (after cell lysis) is below 10% for the acutely oxygenated brain slices. Similar conditions with continuous shaking of the slices on an orbital shaker leads to higher cell lysis. All values represent SEM ± mean, n = 4 experiments. ** $p < 0.01$. B. Silver impregnation of acute brain slices maintained for 8 h in the proposed incubation system with carbogen aeration. Scale bar: 80 µm. C. DiI-stained pyramidal cortical neuron in layer IV. Scale bar: 80 µm. D. Magnified apical dendrite of a cortical neuron with well-visible dendritic spines. Scale bar: 8 µm.

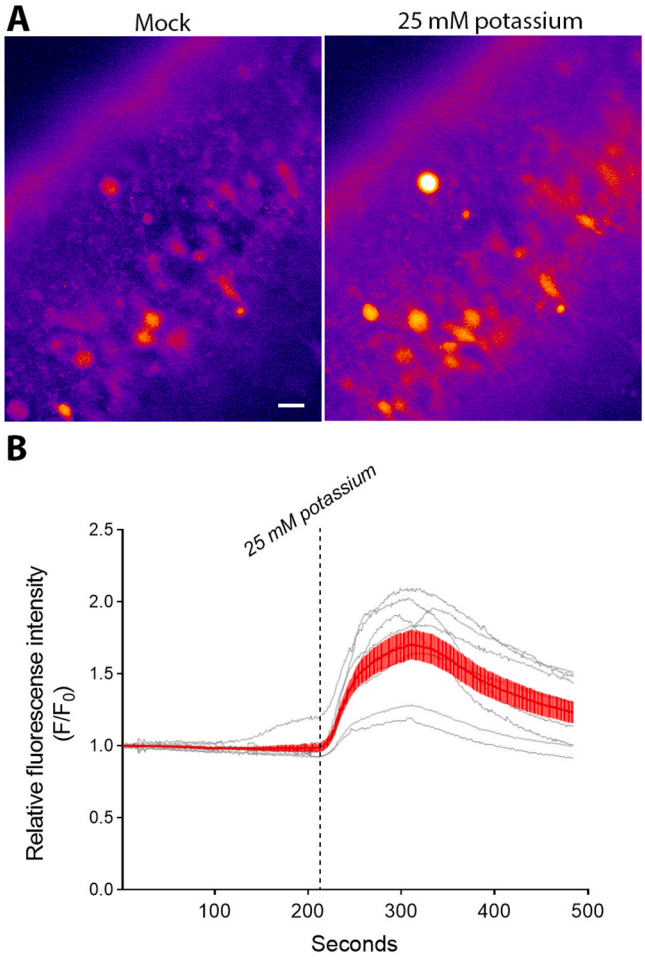

Fig. 4. A. Fluorescent staining with the Cal520 calcium-sensitive dye of hippocampal tissue in the region of CA3. The bodies of the pyramidal neurons are clearly distinguishable. Fluorescence increase after treatment with 25 mM KCl, leading to membrane depolarization, is clearly visible. It affects mostly the bodies of the pyramidal neurons, but also the tissue surrounding the pyramidal neurons demonstrates elevated calcium influx. Scale bar: 100 μm. B. Calcium change curves demonstrate the elevation of the fluorescent signal after exposure to 25 mM KCl to initiate membrane depolarization and subsequent calcium influx, followed by normal physiological restoration of the calcium concentration with the washout of potassium. The experiment is performed in triplicate (3 preparations) with identical results. Data from one representative experiment are presented.

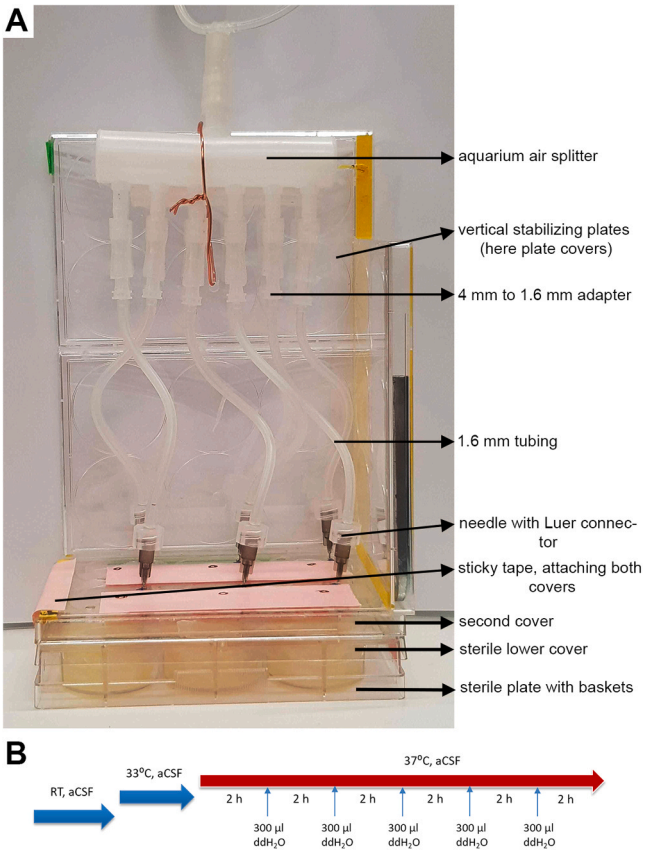

Fig. 5. A. An image of a real-life system with an aquarium air splitter, tubing, vertical plate covers attached with silicon together to improve stabilization of the tubing. Note the sticky tape that attaches both covers together, which provides very good needle stability and minimizes the risk of spontaneous displacement when handling the system. B. A schematic timeline demonstrating the major steps in slice preparation and incubation.

the technical setup in reality with overview of the procedure we used are shown in Fig. 5A, B.

4. Discussion

Our experimental system allows easy and reproducible parallel incubation of multiple treatment conditions involving acute brain slices, using equipment available in every lab. At the same time, the use of sterile aCSF, sterile plates and sterile incubation baskets every time reduces the risk of cross-contamination and the development of resistant bacterial strains, which can compromise any long-term incubation system.

While we do not object the suitability of incubation systems such as Braincubator (Buskila et al., 2014) for longer-term incubations, nor we aim to compare the advantages and disadvantages of different systems, we believe that most shorter-term incubation tasks can be easily performed using our experimental setup. The ability to perform 6 experiments simultaneously, allows to shorten the duration of the experiments studying more conditions at once, which cannot be performed in single condition incubation system, as we have shown on multiple occasions in our previous work (Wippel et al., 2011, 2013; Hupp et al., 2019). Furthermore, this is an opportunity to include a control/replicate from the same animal, minimizing the number of animals having to be sacrificed (Lossi and Merighi, 2018). In everyday experimental conditions, we did not observe the growth of bacteria such as *E. coli* within 12 h of incubation. Additionally, the application of antibiotics in the aCSF (optional) further reduced the risk of contamination. Indeed, antibiotic presence may interfere with various experimental results therefore their application is justified only when proper controls are performed.

The presence of some cell lysis in the acute brain slice system is inevitable due to the cutting though intact tissues. Our previous experiments using this setup demonstrate that within 6 h, massive microglial taxis towards the cutting plane takes place – a clear indication that microglial cells recognize the cutting plane as area of damage (Hupp et al., 2019). The very nature of the acute brain slice system makes it only partially physiologically comparable with the native brain tissue. This is a critical point, which needs to be considered every time when an acute brain slice system is used. The major readout of our experimental setup was the DiI staining and the modified silver impregnation staining, which demonstrated intact neuronal and dendritic configurations. While the DiI staining provided suitable data for confocal imaging analysis, the silver impregnation allowed comparing the slice tissue with intact brain slices from the brains of healthy animals, sliced after sacrifice.

We failed to observe an improvement in the tissue quality after slicing in sucrose or in NMDG-containing medium, as previously

suggested by other groups (Ye et al., 2006; Ting et al., 2014), although we did not see a deterioration either. For our purposes and morphological analyses, aCSF proved an effective environment, however, use of other media might be justified in a different setting. We observed, however, an increased number of dendritic spines if we do not cut on ice, but at room temperature, maintaining the environment properly oxygenated (Eguchi et al., 2020). Indeed, the swelling of the brain slices after cutting them is a known phenomenon (Ting et al., 2014). We believe that our incubation with mild evaporation in the first two hour before replacement of the evaporated fluid with distilled water creates transiently mildly hypertonic environment, which probably counteracts the initial swelling after cutting. An experimental component, which remains obligatory, is the slicing device. We used a vibratome system from Leica, focusing on slice thickness of 340 μm, but alternative system such as tissue choppers can be used as well. In our hands, the vibratome system provided an efficient and reproducible slice preparation.

The application of an immobilization basket was key to our system, because it allowed using oxygenation without mechanical stress on the slices. Brain tissue is highly sensitive to mechanical damage and stress, therefore even mild shaking should be reduced to a minimum (Budday et al., 2020). The presence of a basket allowed easy removal, fixation, washing of the tissue without the need of subsequent slice transfer. The risk of leaking from a well into a neighboring well was also checked using stained medium, as well as bacteria incubation in one well, and analysis of bacterial growth in the neighboring wells. We have never observed leakage from a well into a well, although some care needs to be taken to avoid extreme bubbling and fluid spill out.

In the initial experimental setup, we used only one cover with holes for the needles. This was not effective and precise enough, especially when the cover needed to be taken away and placed back. The placement of two covers with two holes (one exactly below the other) aligned the needles better, allowing it to be placed in stable position. At the same time, the lower cover can be cleaned and/or decontaminated with ethanol, while the second cover serves as a permanent attachment for the air splitter and the tubing.

An interesting modification of the DiI staining we propose here is the application of DiI-coated Tungsten wire (with labeling paste or crystals dissolved in ethanol) instead of the established direct crystal placement (Kim et al., 2007). This provides several advantages – the staining can be precisely applied to specific regions of the brain, and it prevents the overloading with stain, which is a common problem when using DiI crystals. The coating of the Tungsten wire with DiI needs to be performed sparsely to avoid overstating of the tissue.

The verification of the normal morphology of the neurons by DiI and silver impregnation, although informative, was just a snapshot of their condition, but just an indirect marker of normal physiology. Earlier works have demonstrated that the proper physiological function of acute brain slices can be followed either by electrophysiology or by imaging of the calcium influx (for example in the pyramidal neurons of the hippocampus (Ting et al., 2014)). In our system, we followed the pyramidal neurons in the CA3 layer of the hippocampus demonstrating normal calcium influx after KCl depolarization (i.e. preserved membrane potential beforehand) and the expected restoration of the calcium concentration after subsequent potassium washout, indicating preserved physiological ability for removal of the excessive intracellular calcium.

We have used the proposed system in various experimental conditions, focusing on the properties of the cortical neurons in physiology and pathology. Our findings in models of bacterial meningitis and the analysis of pathogenic factors have been confirmed in animals and in human tissues in the similar range as observed in the model, allowing verifying the reliability of the system (Wippel et al., 2013). The disadvantage in comparison with a whole brain in live animals is the access of factors from all sides of a tissue slice (in case of meningitis modeling), while in intact brain all factors penetrate through the cortical surface. Furthermore, as an ex vivo approach the model lacks the influence of macroorganism-specific factors, e.g. adaptive immune responses when using infectious disease models. A general drawback of using slices as a model is the disconnection of the explants from other areas of the brain, which might affect neuroanatomy (Lossi et al., 2009). Careful validation of the model in each individual case is recommended.

The ability to perform multiple treatments at a time is of substantial importance to the experimental animal research concept of 3R – reduction, refinement, replacement (Flecknell, 2002), which raises the awareness of excessive animal use. In this way, researchers can perform simultaneously more experiments reducing the number of controls and utilizing more effectively the available animals.

A thorough cost analysis is beyond our scope (in Switzerland, the total cost of consumables did not exceed 50 CHF per an experiment in a 6-well plate); however, we consider constructing the incubation system (most parts of it which can be subsequently reused) and performing experiments using routinely available equipment and consumables is cheaper by order of magnitudes than acquiring a commercially available system. This could be of interest to scientists in lower income countries, and would generally allow for budget optimizations.

Conflict of interest

The authors declare no competing financial or other interests.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jneumeth.2021.109405.

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