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Making the best of brain slices: comparing preparative methods

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1. Introduction

Participants in the conference were asked to submit their protocols for preparing slices along with the ways in which they evaluated the viability of their preparations. Table 1 was prepared from these responses. The major headings in the table were determined dividing the preparation of slices into 7 steps or choices. These headings are explained in Section 2 below. After the table there is a brief discussion of the evaluation of the health of the slices by each of the investigative groups represented in the table. Following this is a summary of outstanding issues.

2. Explanation of the table headings

2.1. Species and age of the animal

Rats are the animals used unless otherwise indicated. In some cases, ages are chosen deliberately to study particular questions. In other cases, it is a matter of the ease of good slice preparation. In general, the brains of younger animals are easier to dissect free, and the slices survive better than slices from adult animals.

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2.2. Pre-treatment of the animal

In some cases, workers are now treating the animal prior to brain removal in a way designed to reduce the slice damage caused by decapitation and brain dissection. This is a relatively recent development that is becoming more popular in spite of the inconvenience it may pose. Two major treatments use high doses of ketamine and/or an ice-cold transcardial perfusion so that the brain is blood-free and ice-cold when decapitation occurs. The use of an anesthetic is also noted in the table.

2.3. Method of slicing

There are two variables here. The first is whether the hippocampus is isolated before slicing or whether it is maintained within the brain and a hemisphere block then sliced with the hippocampus within the block. This latter may offer some added stability. The second variable is the machine used to do the slicing. Four machines are mainly in use: Vibratome, Vibroslice, McIlwain/Stoelting chopper and Farquhar slicer. The Vibratome is the most difficult to use and requires the most time, but it is generally thought to be the most gentle on the tissue. In some cases slices are made by hand.

2.4. First incubation solution (pre-incubation)

The solutions into which the isolated brain is put and into which the slices are initially placed are made

up of many different compositions. In many cases, these are the same buffers used throughout the experiment, but, in some cases, these are special pre-incubation buffers designed to prevent the slice damage which occurs during this early period. The two most widely used of these are buffers in which most of the NaCl is replaced with sucrose (low Na/sucrose buffer) and buffers designed to prevent Ca- and NMDA-mediated toxicity (anti-NMDA buffers). The latter is often a buffer with low Ca (0 mM) and elevated Mg (10 mM). Another pre-incubation buffer that is used includes a high molecular weight dextran, added to increase colloid osmotic pressure. All of these methods appear to be quite beneficial.

2.5. Buffer composition during incubation

Most experiments are in standard HCO_3 buffers. These are all denoted by ACSF, the general composition of which is provided in the footnotes to Table 1. Calcium concentration is the most variable parameter in these buffers, ranging from 1.2 to 2.5 mM. In most cases, these concentrations have been noted. However, some workers use the high molecular weight dextran and others use antioxidants in the buffer. Both of these appear to be beneficial to slice preservation. Some workers continue to use non-HCO_3 buffers (e.g., Hepes or phosphate) with no CO_2 in the gas. There is no systematic study of the effect of this except that the regulation of intracellular pH is quite different.

2.6. Methods of incubation

Both interface chambers and submerged chambers are used. For the former, there are variants: the Haas-type, where the platform is a ramp, and the type made by FST, where the platform is flat. In some cases there is slow flow, and in other cases the buffer remains static. The submerged chambers tend to be more homemade, and the flow rates vary from 1 to 2 ml/min to 60 ml/min. Overall, the interface chamber appears to yield healthier slices when judged morphologically. However, it is not practical for metabolic measurements.

2.7. Temperatures of incubation

There is a wide variation in the temperatures that are used, from room temperature (21–23°C) to intermediate ranges to physiological temperatures (36°C). This is one of the more controversial areas because a balance must be struck between viability and ease of experimentation, which are optimal at low temperatures, and true physiological properties, which are probably optimal at 35–36°C. Overall, the latter should be used whenever it is at all possible.

3. Outcomes of different preparative methods

Table 1 shows the methodologies used to prepare brain slices by several groups doing research in this field. It is important to be able to evaluate the outcomes of these different treatments in terms of brain slice health. These are described below by each group of workers. Note that in most cases the evaluative criteria are limited to parameters that are actually measured by the particular group. That is, standards are established for accepting slices for experiments. In some cases, there are explicit attempts to evaluate the general health of the slice. These will be discussed at the end of the descriptions. The 4 categories into which all the evaluative techniques fit are as follows: (1) extracellular electrophysiological responses, (2) intracellular electrophysiology, (3) cell morphology (histology), either by light or electron microscopy, and (4) metabolite levels or metabolic processes.

3.1. Specific outcomes as described by the groups

Aitken: electrophysiology. Amplitude of population spike: 5 mV with 150 μA stimulation.

Dudek: electrophysiology. Amplitude of population spike; 15–25 MV; number of cells that can be recorded intracellularly; how long slices can be maintained.

Espanol: metabolite levels. Assessed by NMR. PC/ATP > 1.8.

Ferchmin: electrophysiology. Size of field potentials: 5 mV population spike.

Kreisman: electrophysiology. Population spike larger than 3 mV, no second spike. Onset of population spike should correspond with rising phase of epsp. *Use K-sensitive electrodes:* Extracellular K should be same as in bath. *Histology*: Distinct pyramidal and dentate layers; slice should be amber via transillumination, not gray or dark.

Landfield: electrophysiology. Population spike 4–10 mV in CA1; Intracellular: Rm 40–100 MΩ, RMP −65 mV or more.

Larkman: electrophysiology (intracellular) of motoneurones. Em −55 mV or larger; APs < 1 ms; overshoot 0 mV; evidence for hyperpolarization-activated sag current in response to DC stimulation. Some spontaneous activity. Eight-hours duration. High level of spontaneous activity is a bad sign. *Histology:* In young animals, cells look bright in Nomarski, membrane seems smooth. Note: Concludes that using the sucrose-substituted preincubation buffer greatly improves brainstem slice health.

Leybaert: histology. Trypan blue exclusion.

Lipton: histology. CA1 pyramidal cells very strongly resemble pyramidal cells in situ at both LM and EM levels. This is true for whole population of the pyramidal cells and is verified statistically. *Metabolites:* ATP

levels = 7.7 nmol/mg dry wt; PCr/ATP is 1.5–2. Protein synthesis pattern as in situ. *Electrophysiology:* Extracellular population spikes average 5 mV, extracellular field EPSPs average 4 mV. (At maximal stimulus strengths.) Field potentials and spikes do not vary more than 10% over 4–6 h incubation periods.

Newman: histology. LM. Appearance of CA and DG similar to in situ. Metabolites: ATP levels about 18–20 nmol/mg protein. Wet wt/protein between 11 and 13.

Raley-Susman: metabolites. Protein synthesis pattern resembles in situ. Histology: Cells intact morphologically as seen in frozen sections.

Rice: morphology. In LM cells appear round. *Metabolites:* Extracellular K same as medium. 20% extracellular volume fraction. *Electrophysiology:* striatal slices, field potential of 1 mV.

Santamaria: histology. In LM pyramidal layer looks intact, cells look good.

Sarvey: electrophysiology. One spike only. Paired pulse inhibition: at 20 ms interval there should be between 70 and 100% suppression of second pulse. At 30 ms, it can be between 25 and 50% suppression. This is primarily in DG.

Schurr and Payne: visual appearance under dissecting microscope. Healthy slices are white and opaque; damaged slices are transparent. *Electrophysiology:* a CA1 population spike amplitude > 10 mV with stimulation of twice threshold (4–5 V). No secondary spikes.

Segal: Nothing given.

Sejer and Eskesen: electrophysiology. Field EPSP is 2 mV at 1/2 maximal stimulus.

Taylor: histology. Lightly stained nuclei, compact cell bodies and clearly discernible dendrites.

Teyler: electrophysiology. 'Textbook' waveform, stable baseline for 15–20' with less than 10% drift.

Vasilenko: electrophysiology. Spontaneous activity resembling in situ and also temperature responses that are similar. He points out the importance of temperature effects and recommends working at physiological temperatures.

Veregge: electrophysiology. Single spike with amplitude 2 mV at stimulus intensity less than 300 μA.

Wallis and Panizzon: electrophysiology. Population spike in CA1 3 mV or larger.

Wu and Kelly: histology. 'Good appearance.'

4. Summary and goals for the future: Criteria for healthy slices

Overall, it is clear that the ways in which slices are evaluated still are not standardized. The ultimate goal of a slice preparation is that cell function and cell anatomy in the slice should be the same as in the intact brain. A corollary of this is that slices should be well enough prepared so that experiments can be done at physiological temperatures (36–37°C for rodent slices). This is often not the case, resulting in a very large number of experimental studies being done at subphysiological temperatures.

Clearly, it is not possible to test every variable of cellular function and anatomy. Ideally, a set of criteria for slices should be established, covering morphologic, metabolic and electrophysiologic parameters, and these criteria should be satisfied in order for the results to be acceptable as representative of in-situ-like behavior. These criteria would reflect values of basic cellular parameters, and the implicit assumption would be that if the criteria were satisfied then most functions would probably be similar to that in situ.

For *morphology*, the criteria would be cells that are indistinguishable at the EM level from the same cell types in situ. This is relatively easily assessed, at least in principle.

For *metabolism*, the criteria would be that cells had in-situ-like concentrations of high-energy metabolites and the major cations. It would also mean that cells carried out basic functions at in-situ-like rates: oxidative metabolism, protein biosynthesis.

For *electrophysiology*, the criteria would be that cells show similar resting properties to those in situ and also respond similarly to pathway inputs. The reduction in connectivity that comes from severing afferents and efferents poses a serious problem here. Thus more specific criteria, such as the presence of specific conductances, may have to be established. Minimal levels of population spike and field potentials could be established for both interface and submerged slices.

Overall, this issue is, in fact, a very difficult one to deal with. The brain slice preparation is used very extensively. Most workers have developed preparative techniques that allow them to do the experiments they want to perform. These experiments examine very specific functions, and these functions may well not rely on the complete integrity of the cells. Thus, there are long histories of work using specific preparative techniques, and these are very difficult to jettison if a new preparative technique is indicated. The problem is that there is a real danger here that even the functions being examined are not the same as they are when the tissue is in situ.

It is interesting, in light of the person to whom this meeting was dedicated, that the early workers in brain slices, of whom Henry McIlwain was most prominent, focused almost exclusively on establishing the ionic and metabolic parameters of the cortical slices they were using, trying to assess the similarities and differences between them and in situ tissue. Large numbers of recipes were used to try and produce in-situ-like properties. The results were never completely successful, and the brain slice has persisted in its refusal to be tamed.

Table 1
Preparative methods

Group	Age or weight of animal [a]	Pre-treatment of animal	Method of slicing [b]	Buffer		Type of incubation chamber	Temperature (°C)
				Pre-incubation	Incubation		
Aitken	120–150 g	Ether	Isolated hippocampus, McIlwain. Hemisphere, Vibroslice.	Ice-cold ACSF [c]	ACSF Ca = 1.2	Flat interface; on lens cleaning tissue Flow: 1–2 ml/min	35–36
Dudek	250–400 g	None	Isolated hippocampus, Vibratome or Vibroslice		ACSF Ca = 1.3–2.4	Ramp interface	34 (400) 21 (250)
Espanol	250–300 g	Isoflurane, cool animal, ice-cold transcardial perfusion. Brain at 19°C.	Cortical slice: razor blade		ACSF Ca = 1.2	NMR tube	37
Ferchmin	150 g	None	Hand slicer	Ice-cold ACSF	ACSF Ca = 2	Ramp interface (Stoelting). Three simultaneous lanes	34.5
Kreisman	60–90 days	Ether, ice-cold transcardial perfusion	Isolated hippocampus, Stoelting tissue chopper	ACSF at 21°C for 1.5 to 2 h Interface	ACSF Ca = 2.7	Interface. Flow: 0.6 ml/min	32
Landfield		6 months CO_2 asphyxiation	Isolated hippocampus, McIlwain chopper	None	ACSF Ca = 2	Interface static or flow: 1	33
Larkman	160–250 g (a) or neonatal (n)	None	Brainstem: Vibroslice (a) or DTK tissue slicer at 120 μm (n)	26 mM Na/sucrose ACSF, 37°C (a) 82 mM Na/sucrose; 1 mMCa^{2+}/5 mM Mg^{2+} (n)	Normal ACSF (n and a) Ca = 2	Ramp interface Flow: 0.5 ml/min (a) Submerged (n)	37 (a) 22 (n)
Leybaert	2–4 days postnatal	Ether, cool dorsal region of animal in ice.	Spinal cord; 'embed' in agar: Vibratome 200 μm	Rm temp in interface: HCO_3-buffered Tyrodes	Hepes buffer Tyrode	Submerged	Room temp
Lipton	G.P. * 350 g	None	Isolated hippocampus, Vibratome	ACSF with 0 mM Ca/10 mM Mg. 36°C for 45′	ACSF Ca = 1.3	Submerged: Flow 50 ml/min	36–37
Newman	250 g	None	Isolated hippocampus, Farquhar	ACSF with 3% dextran, chopper m.w. 83,000. 22°C ramp up to 37°C in 15′	Same as pre-incubation buffer: Ca = 1.5	Submerged (1–2 mm)	37
Phillips	200 g	None	Brain stem, striatum. Vibratome	Ice-cold ACSF [c]: can store tissue block up to 3 h – still get normal release of neurotransmitter.	ACSF Ca = 2–2.4	Submerged: Flow 50 ml/min	32–34

Table 1 (continued)

Group	Age or weight of animal [a]	Pre-treatment of animal	Method of slicing [b]	Buffer		Type of incubation chamber	Temperature (°C)
				Pre-incubation	Incubation		
Raley-Susman	60–70 days	None	Isolated hippocampus: Vibroslice or razor blade.	ACSF with 0 mM Ca/10 mM Mg 45′	ACSF Ca = 1.2	Submerged	36
Rice	150–200 g female	Pentobarbital	Hemisphere: Vibroslice or Vibratome thiourea:	ACSF with ascorbate and thiourea 1 h at room temp.	Same Ca = 1.5	Submerged: Flow 1 ml/min	32
Santamaria	200–300 g	None	Isolated hippocampus: McIlwain chopper	ACSF: room temperature	ACSF Ca = 2	Flat interface. Flow 2 ml/min	34
Sarvey	80–180 g	Ketamine (100 mg/kg)	Isolated hippocampus: McIlwain chopper		ACSF Ca = 2.4	Flat interface on tea-bag paper	30–34
Schurr	200–250 g	None	Isolated hippocampus: McIlwain Payne chopper	Ice-cold ACSF	ACSF Ca = 2.5	Flat interface on nylon mesh Flow: 1 ml/min	34
Segal		None	Isolated hippocampus: McIlwain chopper. Use hemisphere with Vibroslice if want better structure. (int.)	ACSF: room temperature	ACSF	Submerged or flat interface	30–32 (sub.) 32–34
Sejer & Eskesen	150–250 g	None	Hemisphere: Vibroslice	None	ACSF	Interface	35
Taylor	200–275 g	Ketamine (200 mg/kg) and ether; transcardial perfusion with ice-cold buffer containing low Na and sucrose with 50 μM ketamine.	Isolated hippocampus: Stoelting tissue chopper	Low Na/sucrose at 4°C. Over next 45′ warm to 31°C.	ACSF Ca = 2	Ramp interface: lens paper. Flow: 1 ml/min	36
Teyler	30–60 days	None	Isolated hippocampus: Stoelting hand slicer	ACSF: room temperature	ACSF Flat interface. Flow: 0.5 ml/min Ca = 2	30–32 Sometimes static – add water steadily to control for evaporation	
Vasilenko	G.P.	None	Brain stem, hypothalamus. Stoelting hand slicer.	None	ACSF [c] Ca = 2.4	Submerged. Flow: 1.5 ml/min	32
Veregge	19–30 days	Metofane	Hemisphere: Vibroslice		ACSF Ca = 2 temp.	Interface	Room
Wu & Kelly	Mse. brainstem	None	Vibroslice	None	ACSF	Submerged	34
Wallis & Panizzon	200–400 g	Halothane	Isolated hippocampus: McIlwain chopper	None	ACSF Ca = 2.4	Submerged: overflow to minimize slice buffeting. Flow: 2 ml/min	34

[a] All animals were rats unless indicated otherwise. G.P. = Guinea pig; Mse. = mouse.

[b] All slices were between 350 and 450 μm unless stated otherwise. All slicing was done into ice-cold buffer unless noted otherwise. Slices were generally manipulated by gently rolling onto a sable brush or by sucking into the large end of a pasteur pipette, maintaining the slice free-floating in the buffer.

[c] ACSF is used to denote a standard buffer with approximate concentrations as follows (in mM): NaCl, 120; $NaHCO_3$, 26; KCl, 3–4; KH_2PO_4, 1.5; $MgSO_4$, 1.4; $CaCl_2$, 1.3–2.4; glucose, 10; equilibrated with 95% O_2/5% CO_2. Significant departures from this composition are noted by inserting any of the concentrations that are different from this.

5. Conclusion

Certain patterns do emerge from the descriptions in this paper.

(1) A large majority of the electrophysiological and transmitter-release studies have been done on slices in which the criteria for health are healthy electrophysiological or transmitter releases. There is a certain tautology here, but these criteria certainly lead to successful experiments. On the other hand, because morphological and metabolic integrities are not established for most of these preparations, results may not reflect in situ phenomena.

(2) Certain explicit efforts have been made to improve slice health These include pretreating the animal with ketamine and/or cooling the animal prior to decapitation. They also include the use of pre-incubating solutions, where the slices are maintained in buffers designed to alleviate damage for relatively short periods after their preparation. A third approach has been to include substances in the normal incubating buffer, either antioxidants or colloids, and maintain these throughout the experiment. Often these efforts are driven by specific goals (e.g., measurement of high-energy phosphate changes, measurement of protein synthesis and measurement of ion movements), and they all have produced results that satisfy certain criteria of cell health. However, much work is necessary to arrive at optimal treatments and to examine enough parameters to allow conclusions about overall slice health.

It is anticipated that the field will eventually become rationalized by an iterative process as different preparative methods become standardized and their effects become more extensively studied.