

Dissection method affects electrophysiological properties of hippocampal slices

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ABSTRACT

The rodent hippocampal slice preparation has long been a critical tool for studying the electrophysiological effects of pharmacological and genetic manipulations. Slices can be prepared with several different slicing methods including the tissue chopper, vibratome, and rotary slicer. To examine how slicing methods affect slice integrity, we generated hippocampal slices by these three methods and compared their histology and electrophysiological responses. Although all three methods generate histological alterations, the time course is slowest in slices generated with a rotary slicer. Furthermore, although paired-pulse facilitation in dendritic field EPSPs was observed in slices generated by all three methods, paired-pulse potentiation in population spikes, which is common in chopper- and vibratome-generated slices was seldom observed in rotary-generated slices, suggesting less disinhibition. For preservation of hippocampal slice integrity, the rotary slicer may offer advantages over the other two devices.

Key words: Hippocampus; slice preparation; paired-pulse facilitation; paired-pulse depression

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INTRODUCTION

Slice preparations have long been a critical tool for assessing electrophysiological and histological effects of pharmaceutical and genetic manipulations in the hippocampus. In addition to hand cutting, there are at least three mechanical slicing devices: tissue chopper, vibratome and rotary slicer. Although hippocampal slices are extensively used, slicing method remains an understudied parameter. Because slices thicker than 700 μm suffer from poor oxygen supply, rodent brain matrices for 1000 μm hand cutting are not practical for preparing viable

tissue. In this work we compared three other methods that are used for cutting hippocampal slices. Of these methods, the chopper was the earliest method developed (Schwartzkroin 1975), while the vibratome is now the most commonly used device (Dingledine et al., 1977). These two methods offer widely different advantages and disadvantages. The chopper is faster and easier to use (Lipton et al., 1995, Wang and Kass 1997), and produces relatively smooth tissue sections. However, the chopper compresses brain tissue, resulting in cell death and

morphological changes (Kaneda et al., 1988, Garthwaite et al., 1979). The vibratome is a more expensive instrument, and because optimal cutting requires a slow advance of the blade, it requires more time to cut, prolonging the time tissue is exposed to highly stressful conditions; this method also can result in serrated sections. The vibratome can also produce brain slices with swelling and a mottled appearance (Kaneda et al. 1988). However, the vibratome is generally thought to more easily detach tissue from the blade and to better preserve tissue structure (Lipton et al., 1995, Bortolotto et al., 2011). Use of the vibratome also keeps tissue submerged in artificial cerebrospinal fluid (ACSF), which keeps the tissue cold and oxygenated (Wang and Kass 1997).

It has yet to be thoroughly investigated if the method of slicing alters hippocampal slice integrity. Some comparisons have found metabolite uptake and preservation to be inferior in chopper-generated slices relative to hand-cutting (Garthwaite et al., 1979) and the vibratome (de Barry et al., 1982). Consequently, some protocols recommend the vibratome over the chopper (Lipton et al., 1995, Aitken et al., 1995, Bortolotto et al., 2011). However, other comparisons between vibratome- and chopper-generated slices did not reveal differences in population spike (PS) amplitude, PS number, of field potentials (Watson et al., 1997), long-term potentiation induction (Taubenfeld et al., 2002), metabolite levels (Whittingham et al., 1984) or oxygen consumption (de Barry et al., 1982). The rotary slicer is a more recent alternative (Kaneda et al., 1986), is quick to use, and, in theory, should cause the least mechanical stress, as discussed later. However, rotary slicers are used by very few groups (Akaneya and Tsumoto 2006, Futatsugi et al., 1999, Kojima et al., 2001, Oh-Nishi et al., 2012, Stein et al., 2014), and these devices are not currently commercially available. Thus, the potential use for the rotary slicer as a cutting method has not yet been established. As differential slicing methods have been used as an explanation for differing results, it is important to critically and thoroughly examine the repercussions of each method.

We recently reported that with all the three slicing methods the immunoreactivity of the dendritic marker Microtubule-associated protein 2 (Map2) declines between two to four hours of incubation period (Stein et al., 2017). However, in the stratum radiatum, we also observed that rotary-generated slices had a nonsignificant, but consistent, trend for higher levels of Map2 immunoreactivity than chopper-generated slices, which had a consistent trend towards higher levels of Map2 immunoreactivity than vibratome-generated slices. Moreover, immunoreactivity of the glutamate transporter VGLUT1 was significantly lower in vibratome-generated sections than in either rotary- or chopper-generated sections (Stein et al., 2017). These findings suggest that the rotary slicer is not only comparable to the vibratome and chopper but may have superior features for preserving slice integrity. Here we compared the electrophysiology and histology of slices prepared by the three cutting devices.

MATERIALS AND METHODS

Animals. All animal procedures were submitted to, and approved by, the Washington University Institutional Animal Care and Use Committee (IACUC), Division of Comparative Medicine, Washington University School of Medicine, St. Louis, MO, and were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23). All efforts were made to minimize the number of animals used and their suffering.

Hippocampal slice preparation. Hippocampal slices were prepared from postnatal day 34 ± 1 Sprague-Dawley rats purchased from Harlan (Indianapolis, IN) by L.R.S. and Y.I. using standard methods (Stein et al. 2014). Rats were moved to the dissection room the night prior to sacrifice and dissections were performed between 10:30 am and 12:30 pm to account for possible effects of transportation and the light/dark cycle, respectively. Since slice viability is closely linked to experimenter experience and expertise, Y.I. and C.F.Z., who have been publishing on slice electrophysiology for over 30 years, trained L.R.S. in identical technique. Briefly, rats were anesthetized with isoflurane and decapitated. Hippocampi were dissected, carefully freed of the pia mater, and placed in 30°C artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 5 KCl, 2 MgSO₄, 2 CaCl₂, 1.25 NaH₂PO₄, 22 NaHCO₃, 10 glucose, gassed with 95% O₂-5% CO₂ and sectioned transversely into 500 µm slices. This thickness was chosen to allow comparisons with previous protocols (Whittingham et al. 1984) and to minimize the cellular swelling that occurs in thicker slices (Bak et al. 1980). Although slicing hippocampi at 4°C rather than 37°C does not affect metabolic profiles and electrical responses (Whittingham et al. 1984), sectioning by vibratome and rotary slicer was done in 4-6°C ACSF. The chopper and vibratome were used according to standard protocols (Wang and Kass 1997). To eliminate possible bias caused by changes in solution pH, dissolved oxygen, and temperature, the same number of samples were subjected to each technique on each dissection day. All slices were from the dorsal hippocampus.

For the tissue chopper (Brinkmann Instruments), we placed hippocampi on filter paper perpendicular to the blade and finished slicing in less than 1 minute. The chopped hippocampal slices were immediately transferred to ACSF. The chopper was used with a Personna Super double edge stainless steel razor blade (0.11 mm thick and 0.33 mm blade wide) and a cutting angle of 12° to 15°. Different from other devices, the cutting angle of the chopper is independent from the blade proceeding speed.

For the vibratome (Vibroslice SYS-NYSLM1), we mechanically stabilized hippocampi by pinning them vertically in a channel carved into a 3% round agar block placed in a bath of ACSF; slicing took 5 to 10 minutes. The vibratome was used with a Valet blade (0.3 mm thick with a bevel 1.0 mm wide) at a half maximal vibratome speed of 2,250 rpm, which equates to 75 mm/s. The settings we

used had the vibratome blade shake at 0.07 to 10 mm/s. The cutting angle for the vibratome ranges from 0.1° to 17°.

For the rotary slicer (made by Dr. Hiroshi Kato, Yamagata University), we pinned hippocampi horizontally in a channel carved into a 3% round agar block placed in a bath of ACSF. Slicing was completed in 3 to 5 minutes. In the rotary slicer, the cutting angle is sharper than the bevel angle. When the blade does not rotate, the bevel angle is the cutting angle. The faster the blade rotation or the slower the speed with which the blade descends into the tissue, the sharper the cutting angle. We used the rotary slicer with an Olfa RR28 rotary blade (28 mm diameter, 0.3 mm thick, 1.0 mm wide bevel). The edge angle was 17° edge angle while the real cutting angle was 0.07° ($\tan = 0.3/28 \times 3.14 \times 3$) when the blade rotated 9 times/s, traveling 792 mm/s ($\tan^{-1}(0.3\text{mm}/792\text{mm})$). For electrophysiology, slices were incubated in gassed ACSF for 2 hours at 30°C before experiments.

Histology. Hippocampal slices were fixed in phosphate-buffered solution of 4% paraformaldehyde (PFA) overnight, equilibrated in 15% sucrose overnight, equilibrated in 30% sucrose overnight, frozen, and stored at -80°C until sectioning. The first and last 100 μm of the 500 μm sections were discarded. The inner 300 μm of each slice was cut into 30 μm sections in a 1 in 4 series were made by cryostat and stored at -30°C in cryoprotectant until use. Hematoxylin and eosin (H&E) staining was performed by the Washington University School of Medicine Histology Core according to standard techniques. For H&E staining and immunohistochemistry (next section), staining protocol bias was avoided by mounting treatment groups together across multiple slides.

Hippocampal slice physiology. Experiments were performed in a submersion-recording chamber at 30°C with continuous perfusion of ACSF (2 ml/min), using previously described and validated standard methods (Tokuda et al., 2010, Stein et al. 2014). Briefly, extracellular recordings were obtained from the CA1 apical dendritic

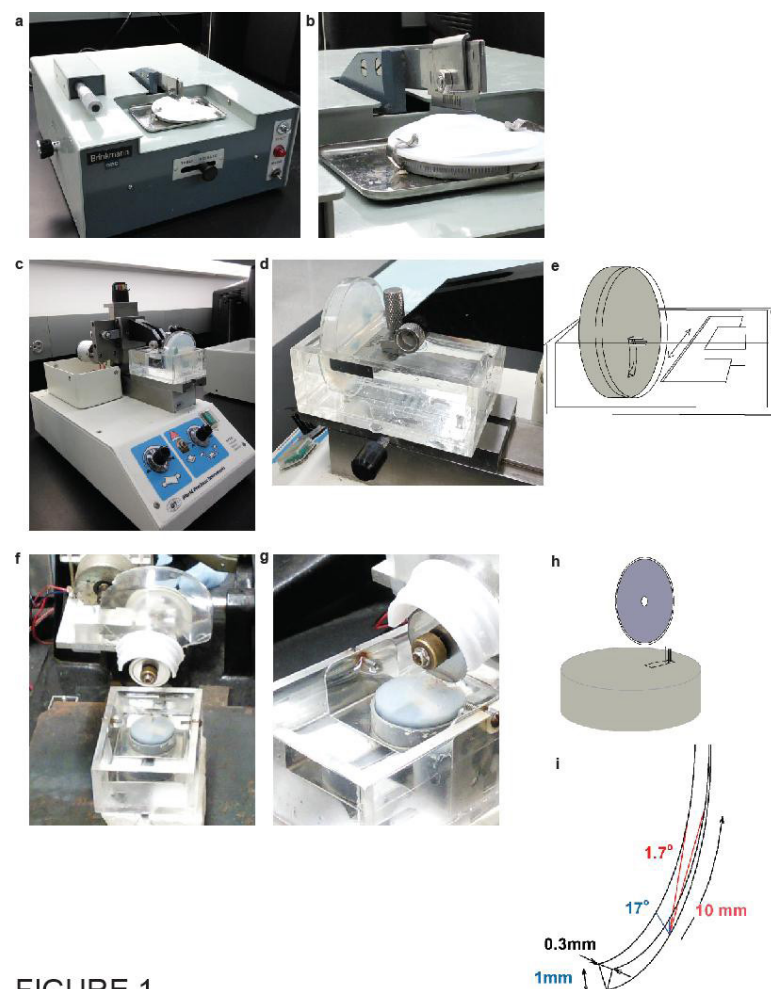


FIGURE 1

Figure 1. Schematic highlighting key features and differences among slicing methods. Acutely isolated hippocampi from postnatal day 33 to 35 Sprague-Dawley rats were sliced by chopper (A,B, Brinkmann instruments), vibratome (C,D, Vibroslice SYS-NYSLM1), or rotary slicer (E,F,G, made by Hiroshi Kato). Currently rotary slicer is not commercially available (Model DTY 7700, Dosaka Co., Ltd, Osaka, Japan) but it is possible to compose it by mounting a DC motor on a rotary microtome. G, In the rotary slicer, the cutting angle is sharper than the bevel angle. A rotary blade with a 1 mm bevel and 0.3 mm thickness has a bevel angle of 17°. When the blade does not rotate, the bevel angle is the cutting angle (blue). When the blade rotates and travels 10 mm down, the cutting angle is 1.7° (red).

region (stratum radiatum) for analysis of field excitatory postsynaptic potentials (fEPSPs) using 2 M NaCl glass electrodes (5–10 M Ω). Responses were elicited with 0.1 ms constant current pulses through a bipolar electrode in the Schaffer collateral pathway. fEPSPs were measured by the maximal slope of their rising phase. PS amplitude was measured as the difference between the initial peak and deepest point whereas somatic fEPSP height was measured as the absolute height of the second peak after PS (or the peak without PS) from the baseline (Fig. 2c). An input-output curve was obtained to determine stimulus intensities for subsequent analyses. Input-output curves were generated using stimuli of 8 different intensities to allow determination of half maximal responses, as described (Stein et al. 2014).

For investigation of paired-pulse plasticity, dual stimuli of identical intensity were delivered at an interval of 21 ms as described in detail (Izumi et al. 2007; Murayama et al. 2006; Nagashima et al. 2005). Briefly, the stimulus intensity was initially set below threshold for evoking responses and increased in a step-wise fashion every 10 sec until 6 pairs of stimuli were administered. The smallest stimulus was set to evoke a response less than half maximal while the largest stimulus was designed to evoke a fully saturated response based on prior experience (Izumi et al. 2007; Murayama et al. 2006; Nagashima et al. 2005). The stimulus intensity needed to evoke a half maximal PS by the first stimulation was determined by the intersection of the horizontal 50% maximal line with a line connecting the two points straddling the half maximal value. This value was used to calculate the size of dendritic fEPSP required to generate a PS of this magnitude. We next calculated the stimulus intensity required for the second (paired) stimulus to induce an equivalent fEPSP. At this stimulus intensity, we could then determine the PS amplitude generated by the second stimulation.

Statistical Analyses. Numerical data are presented as mean \pm standard error of the mean (S.E.M.). Statistical significance was determined by Analysis of Variance (ANOVA). Statistical analyses were performed using R or SigmaPlot 5.01 and 9.0 and SigmaStat 3.1 (Systat Software Inc., Richmond, CA). *P*-values of less than 0.050 were considered significant. Sample sizes are stated in figure legends and refer to individual rats.

RESULTS

Hippocampal slices generated by tissue chopper, vibratome, and rotary slicer are electrophysiologically responsive.

To test the effects of incubation period on slice health, we acutely isolated hippocampi from postnatal day 33 to 35 Sprague-Dawley rats. Previous work had shown that hippocampal slices generated by mechanical chopping and manual slicing differed substantially in their degree of preservation after incubation (Garthwaite et al. 1979). Thus, to further determine if slicing method modified the

effect of incubation period on hippocampal slice health, we generated hippocampal slices by tissue chopper, vibratome, and rotary slicer. In all slicing techniques, the dentate gyrus was placed face down at the time slices were cut.

To verify that our slices were electrophysiologically responsive, we tested maximal dendritic fEPSPs, somatic fEPSPs, and PSs. All slices yielded good responses. To more completely probe for differences, we assessed input-output curves and paired-pulse ratios in slices 2 hours post dissection by delivering two identical stimuli at an interval of 21 ms. We selected the 2 hour time point because it allows sufficient time for slices to recover fully from dissection electrophysiologically and metabolically (Whittingham et al. 1984). The paired-pulse ratio of dendritic fEPSPs is a measure of presynaptic short-term plasticity, and describes the ability of synapses to change neurotransmitter release on the second of two closely spaced afferent stimulations. A response to the second stimulus smaller than the response to the first is paired-pulse depression whereas a response to the second stimulus larger than the response to the first stimulus is paired-pulse facilitation. Under the ionic conditions used, healthy slices show paired-pulse facilitation of dendritic fEPSPs and paired-pulse depression of PS (Tokuda et al. 2010).

We first generated input-output (IO) curves from 8 different stimuli and calculated the size of the fEPSP required to generate half-maximal PS following the first and second paired stimuli (Fig. 2B,C). We determined the stimulus intensity needed to evoke a half maximal fEPSP by the first stimulation using the intersection of the horizontal 50% maximal line (Fig. 2B, blue horizontal arrow in the lower graph) with a line connecting the two points straddling the half maximal value. The second fEPSP evoked by the same intensity stimulus was bigger than the first fEPSP (Fig. 2B, blue upward arrow) indicating paired pulse facilitation in dendritic fEPSPs. We then determined the stimulus intensity needed to evoke a half maximal PS by the first stimulation again using the intersection of the horizontal 50% maximal line (red horizontal arrow in the upper graph) with a line connecting the two points straddling the half maximal value (a). This value allowed us to calculate the size of dendritic fEPSP required to generate a PS of this magnitude (the intersection of the downward red arrow and the solid black line connecting two points (black circles) on the initial fEPSP IO curve, b). We next calculated the stimulus intensity required for the second (paired) stimulus to induce an equivalent fEPSP [horizontal red arrow intersecting the line from the second pulse [gray circles] fEPSP curve, c). At this stimulus intensity, we determined the PS amplitude generated by the second stimulation (red upward arrow intersecting the line on the second PS [gray circles] curve, d). This analysis showed that the PS generated by a second stimulation at an equivalent fEPSP size was much smaller than the PS produced by the initial stimulus, indicating paired-pulse depression. Similarly, green arrows between

middle and lower graphs show that the somatic fEPSP generated by a second stimulation at an equivalent fEPSP size is much smaller than the somatic fEPSP produced by the initial stimulus. However, the PS generated by a second stimulation at an equivalent somatic EPSP size was not smaller than the PS produced by the initial stimulus (purple lines between upper and middle graphs), implying less paired pulse alteration in somatic firing.

Using the analysis outlined in the previous paragraph, we assessed the effects of the paired-pulse paradigm on PSs. Although paired-pulse facilitation was observed in PS height in all slices cut with the chopper (121±28%) and vibratome (136±34%), paired-pulse facilitation in PS height was only seen in 2 of 8 slices cut with the

rotary slicer (80±30%, Fig. 2D). The paired-pulse ratio of the PS height calculated from somatic fEPSPs was also not depressed in vibratome- (114±22%) and in rotary- (118±23%) generated slices, but this effect was less clear in chopper-generated slices (89±21%, Kruskal-Wallis one way rank, $p=0.213$, Fig. 2E). In contrast to the effect of somatic fEPSPs on PS height, the PS height produced by dendritic fEPSPs resulted in paired-pulse depression in slices prepared by all three methods. However, paired-pulse depression was more robust in slices cut with the rotary slicer (17±9%, ANOVA, $[F(2,21)=4.00, p=0.034]$, Fig. 2F) than in slices cut with the vibratome (80±18%, TukeyHSD post-hoc, $p=0.050$) or chopper (76±23%, TukeyHSD post-hoc, $p=0.068$).

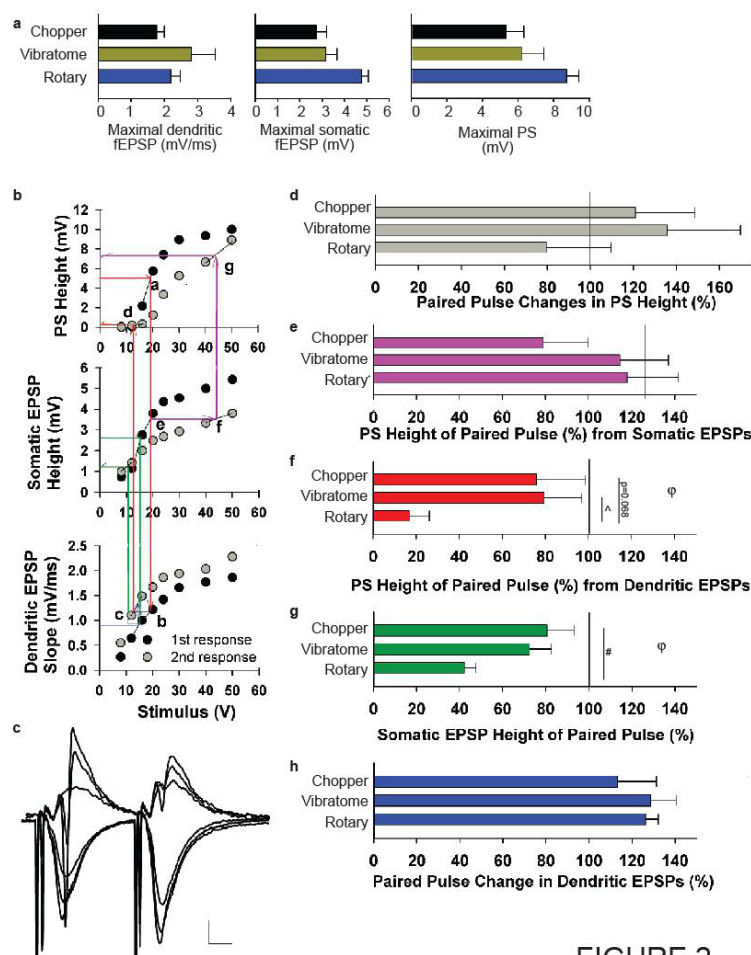


FIGURE 2

Figure 2. All hippocampal slices were electrophysiologically responsive regardless of slicing method. Hippocampal slices generated by tissue chopper (n=7), vibratome (n=7), or rotary slicer (n=8) were assessed after 2 hours of recovery. **A**, Maximal dendritic fEPSPs (left), somatic fEPSPs (middle), and PSs (right). **B**, Typical input-output (IO) curves for PS height (upper), somatic fEPSP height (middle) and dendritic fEPSP slopes (lower) evoked by paired pulse stimuli delivered at an interval of 21 ms in a rotary-generated slice. Black circles represent responses evoked by the first stimuli. Gray circles represent responses evoked by the second stimuli. Red arrows between upper and lower figures show methods to assess paired pulse alteration of PSs. See text for details of calculations and explanation of arrows. **C**, Representative PSs and fEPSPs evoked by 3 different paired pulse stimuli in the same slice. Calibration: 1 mV, 5 ms. **D**, The second PS height evoked by the same intensity stimulus to induce the half maximal initial PS. **E**, The PS height generated by a second stimulation at an equivalent somatic fEPSP size. **F**, The PS height generated by a second stimulation at an equivalent dendritic fEPSP size. **G**, The somatic fEPSP height generated by a second stimulation at an equivalent dendritic fEPSP size. **H**, The second dendritic fEPSP slope evoked by the same intensity stimulus to induce the half maximal initial fEPSP slope. Data are presented as mean ± S.E.M. ϕ represents a significant effect generated by ANOVA. #, ^ represent post-hoc significance between chopper and rotary, and rotary and vibratome, respectively. $\phi, \#, ^ P < 0.05$.

Next, we examined the effects of paired-pulse stimulation on dendritic fEPSPs. Paired-pulse depression was seen in somatic fEPSP height in slices generated by all three methods. However, the paired-pulse depression seen in chopper- ($81\pm 13\%$) and vibratome- ($73\pm 10\%$) generated slices was less robust than the paired-pulse depression observed in slices cut with the rotary slicer ($42\pm 5\%$, ANOVA, $[F(2,21)=4.81, p=0.020]$, Fig. 2G). Post-hoc tests revealed statistical significance for the difference between rotary- and chopper-generated slices (TukeyHSD post-hoc, $p=0.023$). In contrast, paired-pulse facilitation in dendritic fEPSPs was observed in slices generated by all three methods (chopper: $113\pm 18\%$; vibratome: $129\pm 12\%$; rotary: $126\pm 6\%$, Fig. 2H). Thus, while slices generated by all three methods were electrophysiologically responsive, chopper- and vibratome-generated slices exhibited reduced paired-pulse depression of PS height induced by dendritic fEPSPs and reduced paired-pulse depression of somatic fEPSP height relative to rotary-generated slices.

Pyramidal cell layer morphology and dendritic integrity are rapidly affected by slice incubation

We next examined the histological appearance of slices under each condition by hematoxylin and eosin (H&E) staining of cryosectioned slices. Immediately after

dissection, the morphology of CA1 pyramidal neurons was similar among slicing methods (Fig. 3B). However, 2 hours post dissection, cellular shrinkage and dark, pyknotic nuclei were observed. These effects were exacerbated at 4 hours post dissection.

When we separated the damage by slicing technique, we noticed some interesting differences. After 2 hours there was more damage in vibratome-generated sections than in sections generated by the other methods. By 4 hours post dissection, chopper-generated slices had the worst appearance, with a condensed pyramidal cell layer composed almost entirely of pyknotic cells surrounded by empty spaces, as previously reported in chopper-generated slices (Garthwaite et al. 1979) and in slices that are very thick ($700\ \mu\text{m}$) (Bak et al. 1980). At 4 hours post dissection, vibratome-generated slices showed these same traits, but to a lesser degree. On the other hand, rotary-generated slices showed fewer empty spaces, fewer pyknotic cells, and less condensation of the pyramidal cell layer.

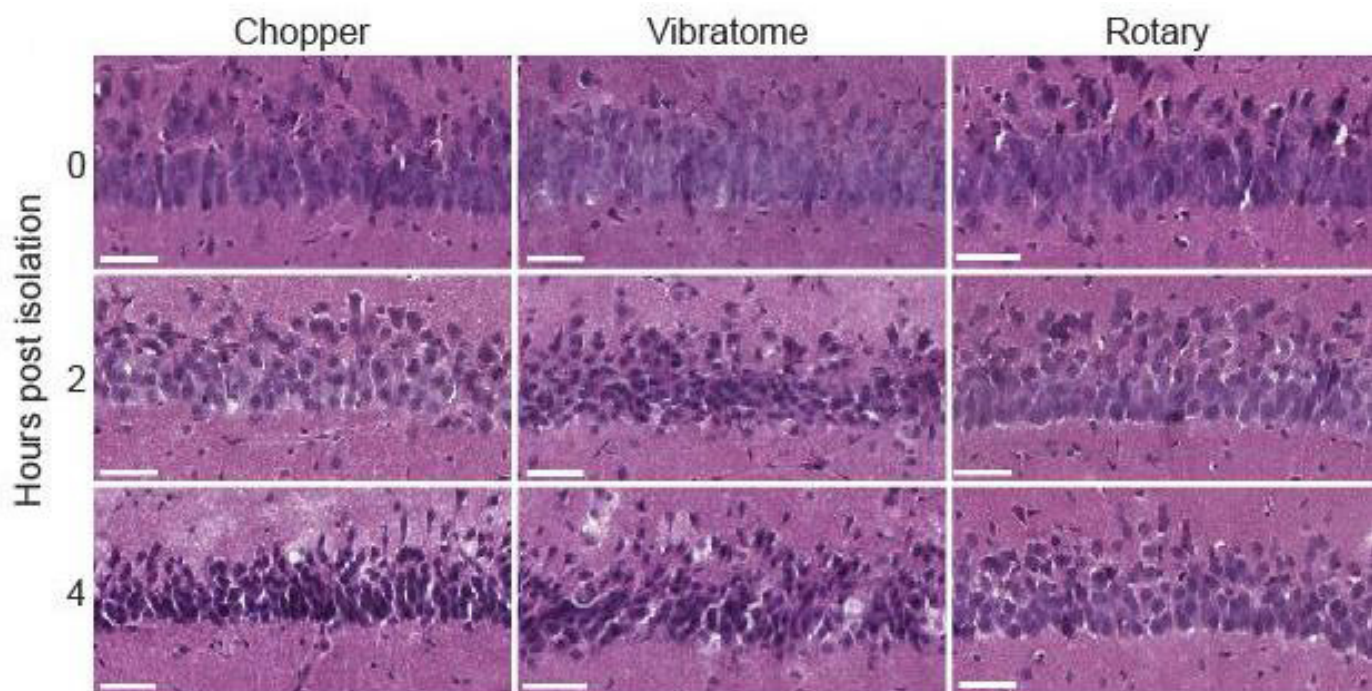


Figure 3. H&E staining of hippocampal slices after 0, 2, and 4 h of recovery in ACSF generated by the tissue chopper, vibratome, and rotary slicer ($n=4$). Scale bars represent $50\ \mu\text{m}$.

DISCUSSION

Here, we report a morphological and electrophysiological comparison of chopper-, vibratome-, and rotary-generated hippocampal slices. We previously reported that slicing method differentially affected Map2 and VGlut1 immunoreactivity, and microglial numbers. In the present study, slicing method did not differentially affect maximal dendritic and somatic fEPSPs and PS heights, paired-pulse changes in dendritic fEPSPs, but had a significant differential impact on paired-pulse PS height, paired-pulse somatic fEPSP height, and tissue morphology. Overall, rotary-generated slices displayed characteristics superior to chopper- or vibratome-generated slices, as initially found by Kato's group (Kaneda et al., 1986). The finding that slicing method differentially affects hippocampal slice morphology and electrophysiology has important implications for studies using the hippocampal slice model system. It was also unexpected since manipulation of many other variables has little effect on slice viability (Whittingham et al., 1984, Leonard et al., 1991).

Our finding that chopper-, vibratome-, and rotary-generated slices are electrophysiologically responsive is consistent with previous work that assessed long-term potentiation at the Schaffer collateral CA1 synapse in chopper- and vibratome-generated slices (Taubenfeld et al., 2002).

The paired-pulse ratio is inversely correlated with probability of transmitter release, such that synapses with low probability of release show paired-pulse facilitation, whereas synapses with high probability of release show paired-pulse depression (Thomson 2000, Stevens and Wang 1995). However, it should be noted that the paired-pulse ratio in somatic EPSPs and that in dendritic EPSPs are not necessarily parallel. In this study, paired-pulse facilitation in dendritic field EPSPs was observed in slices generated by all three methods (Fig. 2H) but paired-pulse depression was observed in somatic EPSPs (Fig. 2G). In particular, the paired-pulse depression of somatic EPSPs is most readily apparent in rotary-generated slices. Accordingly, the paired-pulse ratio of population spike height adjusted from dendritic EPSPs is apparently depressed in rotary slice generated slices (Fig. 2F). The paired-pulse depression in somatic EPSPs suggests alteration of dendritic propagation. It is possible that rotary slicing better preserves dendritic integrity and signal propagation compared to the other slicing methods. In this study, we used a 21 ms paired-pulse interval to characterize synaptic facilitation and/or depression, and attributed changes to presynaptic mechanisms. However, at 21 ms, GABAergic inhibition may contribute to population spike amplitude (Nagashima et al. 2005; Nathan et al. 1990). Thus, the change in PS upon paired stimulation actually reflects monosynaptic depression and facilitation at excitatory terminals, activation of inhibitory interneurons, efficacy of inhibitory synapses, and excitability of interneurons and pyramidal cells.

What accounts for the differences in physiology and morphology among the cutting methods? We suspect that the differences in physiology and morphology result from differences in vibrations and cutting angles during slice preparation; the rotary slicer's absence of vibrations and lower cutting angle may contribute to its apparent benefits. The cutting angle of the chopper is identical to the bevel angle. In contrast, the cutting angle of a vibratome varies 0.1 to 17°, and that of a rotary slicer is 0.02° when a blade proceeds at a speed of 1 mm/sec (see the Methods section and Fig. 1I for details). The lower cutting angle of the rotary slicer may generate cleaner cuts. Thus, it is intuitive that Map2 and VGlut1 immunoreactivity are more vulnerable to the chopper and vibratome than the rotary slicer as recently reported (Liana et al., 2017). The chopping motion of the chopper may compress the tissue whereas the vibration of the vibratome may shred the tissue. On the other hand, the curved angle of the rotary blade enables cleaner cuts with less force on the tissue. The main advantage of chopper-generated slices, and major difference among these three methods, is the speed of slicing. The chopper is the fastest, taking less than 1 minute, the vibratome is the slowest, taking over 5 minutes, and the rotary slicer is intermediate, taking 2 to 3 minutes. As long as slices are kept cold, speed may not be critical as the speed associated with chopper-generated slices did not equate to phenotypic improvement in our assessments. In support of this idea, other work has found that viability was the same between 5 and 30 minute delays and that postmortem delay of up to 3 h do not impair hippocampal field potentials evoked from *in vitro* slices obtained by chopper (Leonard et al., 1991). However, slow slicing would not be an advantage in approaches in which slices are cut at physiological temperatures (Ankri et al., 2014).

In sum, we have compared two standard and one relatively novel slicing protocol used to prepare hippocampal slices. All three hippocampal slice methods quickly result in histological alterations even in the presence of preserved electrophysiological responses. However, the time course of the histological alterations appears to be fastest in vibratome-generated slices and slowest in rotary-generated slices. Moreover, rotary-generated slices performed the best in paired-pulse paradigms. Thus, we propose that the rotary slicer should be considered as an alternative device for slice preparation with results that are equivalent to, or perhaps better than, the other two standard devices.

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DISCLOSURES

C.F.Z. serves on the scientific advisory board of Sage Therapeutics. L.R.S and Y.I. declare no competing financial interests.

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