

2009

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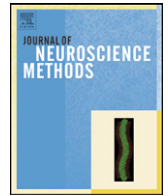
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Recommended Citation

Buerlein, R. C., Hyde, T. M., Lipska, B. K., Robinson, W., Khosla, A., & Kleinman, J. E. (2009). A comparison of human brain dissection by drill versus saw on nucleic acid quality. *Journal of neuroscience methods*, 179(1), 68-70.

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Short communication

A comparison of human brain dissection by drill versus saw on nucleic acid quality

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ARTICLE INFO

Article history:

Received 9 December 2008

Received in revised form 24 December 2008

Accepted 29 December 2008

Keywords:

DNA

RNA

Postmortem

Dissection

Cerebellum

ABSTRACT

This study examined the effect of two dissection techniques on the quality of human brain specimens. Frozen cerebellar samples were obtained from postmortem brains of 10 subjects free from neurological and psychiatric disease. These tissues were tested for RNA and DNA concentration and quality after being dissected with either an electric dental drill or a small handsaw. RNA and DNA were extracted separately from each sample, and the concentrations and quality of each were measured. We found that dissection technique does not significantly affect RNA or DNA quality/yield. RNA and DNA yields, as well as RNA integrity showed no significant differences between the two dissection techniques. Therefore, these results support the use of a high-speed hand-held electric dental drill as an efficient and anatomically precise means of human brain dissection without compromising tissue quality.

Published by Elsevier B.V.

1. Introduction

Many scientific studies require the use of postmortem human brain tissue, so high-quality tissue is critical to obtaining accurate and reliable results (Fleige and Pfaffl, 2005; Lipska et al., 2006; Schroeder et al., 2006). In most laboratories, after the collection of a postmortem human brain, each hemisphere is cut into coronal slabs and then flash-frozen. For experimental analyses, selected slabs are carefully dissected to isolate regions of interest. Although the use of a small handsaw has served as a traditional, effective tech-

nique for human brain dissection, it is time-consuming and often anatomically imprecise. Tourtellotte et al. introduced the technique of using a high-speed hand-held dental drill for the dissection of frozen human brain tissue (Tourtellotte et al., 2003). On account of this, some have switched to the use of a hand-held electric dental drill, which produces more precise, cleaner cuts with high efficiency (Tajouri et al., 2003). Some critics have argued that while the dental drill may be a more efficient technique for dissection, it may generate heat, thus damaging the dissected tissue and compromising RNA and/or DNA quality (Whalan, 2006; Xu et al., 2007).

The present study examined the effects of two dissection techniques on the quality of extracted RNA and DNA to determine if the use of an electric dental drill is an efficient and anatomically precise means of dissection without compromising tissue integrity. The results of this investigation will allow researchers, who use post-mortem human tissue in their studies, to choose an appropriate, reliable method of tissue dissection that does not compromise RNA or DNA quality.

2. Materials and methods

2.1. Human postmortem tissue collection and dissection

At the Clinical Brain Disorders Branch, frozen cerebellar samples were obtained from postmortem brains of 10 subjects free from neurological and psychiatric disease with informed consent from the legal next of kin (protocol #90-M-0142 approved by

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the NIMH/NIH Institutional Review Board) at autopsy from the Washington, DC and Northern Virginia Medical Examiners' Offices. Clinical characterization, diagnoses, and macro- and microscopic neuropathological examinations were performed on all cases using a standardized paradigm (Lipska et al., 2006). Uniform blocks of frozen brain tissue (size 0.5 cm high × 0.5 cm wide × 0.5 cm long), stored at -80°C , from the lateral aspect of the left superior cerebellar hemisphere, were dissected in the coronal plane using either a hand-held high-speed dental drill (Cat UP500-UG33, Brasseler, USA) or a handsaw (Nicholson 80170 No. 50 Coping Saw with blade size 6.75 in. long × 1/8 in. wide). All brain tissue used in this experiment came from subjects who were free of a history of neurological and psychiatric illness or significant alcohol or drug abuse.

2.2. Tissue retrieval and processing: RNA and DNA extraction

Cerebellums were separated from the underlying brainstem by transecting the cerebellar peduncles, cut into two equal slabs parallel to the longitudinal axis of the brainstem, flash frozen and stored at -80°C . Total RNA was extracted from ~100 mg of tissue using the Qiagen RNeasy[®] Lipid Tissue Midi Kit (Cat No. 74804, Qiagen, Valencia, CA, USA). Total DNA was extracted from 100 to 140 mg of tissue using the Genra Puregene Cell Kit (Qiagen, Valencia, CA, USA).

2.3. Concentration and RIN measurements

RNA and DNA concentrations were measured in triplicates using the NanoDrop ND-1000 Spectrophotometer V3.2.1 using 1.5 μL of sample. RNA Integrity Numbers (RIN) were obtained by following the protocol outlined in the Agilent 2100 Bioanalyzer with Agilent 2100 Expert software (Part No. G2940CA Agilent Technologies, Palo Alto, CA) using Agilent RNA 6000 Nano Kits (Part No. 5067-1511 Agilent Technologies, Palo Alto, CA).

2.4. DNA quality assessment

DNA quality was assessed by running the samples on a Reliant Gel System with 2% SeaKem Gold Agarose gel with 1× TE Buffer (pH 8.0, Quality Biological Inc., Cat. No. 351-011-131) and ethidium bromide (Cat. No. 54939, Lonza, Switzerland). The gel was run at 200 V for 30 min on gel electrophoresis equipment (Bio-Rad PowerPac Basic, Cat. No. 164-5050, Bio-Rad Laboratories, Hercules, California). A Trackbit 1 kb DNA Ladder was used (Cat. No. 10488-72, Invitrogen). DNA samples were prepared and loaded using a 6× Loading Dye (Cat. No. R0611, MBI Fermentas). The gel was visualized using the Kodak EDAS 290 High-Performance Ultraviolet Transillu-

Table 2
DNA concentrations and yield.

Sample #	Age (years)	Saw		Drill	
		Concentration (ng/ μL)	DNA yield	Concentration (ng/ μL)	DNA yield
1	48	764.6	3.86	501.5	2.69
2	31	762.8	3.85	828.3	4.83
3	27	286.4	1.58	734.9	3.27
4	46	810.0	4.19	647.8	2.78
5	49	823.7	3.53	220.7	1.30
6	30	739.1	3.41	658.5	2.93
7	42	564.0	2.66	564.2	2.84
8	45	497.4	2.66	927.3	4.49
9	38	728.8	3.90	764.4	3.99
10	42	184.1	1.10	464.6	2.03
Mean	39.8	616.1	3.07	631.2	3.11
S.D.	7.94	227.1	1.05	203.7	1.08

Table showing age of subjects (years), DNA concentrations (ng/ μL) and yield of DNA (expressed as μg of DNA per mg of tissue used for extraction) from the tissue samples dissected with either the hand-held saw or the high-speed dental drill.

minator with a 302-nm UV source (Ultraviolet Laboratory Products, Upland, California).

2.5. Statistical analysis

Two-tailed student's *t*-tests were performed to assess statistical differences in the RIN and yields of RNA and DNA. Statistical analyses were performed using Statistica (StatSoft Inc., STATISTICA (data analysis software system) version 7.1 www.statsoft.com).

3. Results

There were no significant differences between the yield of RNA ($t = -0.061, p = 0.95$) or DNA ($t = -0.081, p = 0.94$) for the two methods of dissection (see Tables 1 and 2). The quality of the extracted RNA also did not differ between the two dissection methods, comparing RIN values ($t = -0.99, p = 0.33$, see Table 2). The presence of a single clear band and the absence of additional bands or streaking of the samples on agarose gels indicated that the quality of the DNA did not differ between the two techniques (see Fig. 1).

4. Discussion

RNA and DNA yields and quality were measured for both methods of tissue dissection, and no significant differences were observed. The purity and integrity of the RNA samples were assessed using an Agilent 2100 Bioanalyzer, and the results are

Table 1
RNA concentrations, yield and RIN.

Sample #	Age (years)	Saw			Drill		
		Concentration (ng/ μL)	RNA yield	RIN	Concentration (ng/ μL)	RNA yield	RIN
1	48	1033.2	0.71	8.3	1076.2	0.80	8.3
2	31	1171.6	0.88	8.5	1210.0	0.84	8.5
3	27	855.9	0.62	8.3	991.6	0.75	8.4
4	46	1107.4	0.81	8.5	907.7	0.70	8.7
5	49	1169.8	0.81	8.2	1115.8	0.76	8.1
6	30	1066.4	0.80	8.5	1085.1	0.73	8.6
7	42	1219.6	0.89	8.5	1113.4	0.84	8.2
8	45	923.0	0.67	8.4	1074.0	0.74	8.6
9	38	1015.6	0.80	8.6	1218.6	0.84	8.8
10	42	1091.9	0.80	8	1102.2	0.82	8.5
Mean	39.8	1065.5	0.78	8.38	1089.4	0.78	8.47
S.D.	7.94	113.6	0.09	0.18	91.7	0.05	0.22

Table showing age of subjects (years), RNA concentrations (ng/ μL), yield of RNA (expressed as μg of RNA per mg of tissue used for extraction) and the RIN values obtained for each RNA extraction (RIN = RNA integrity number) from the tissue samples dissected with either the hand-held saw or the high-speed dental drill.

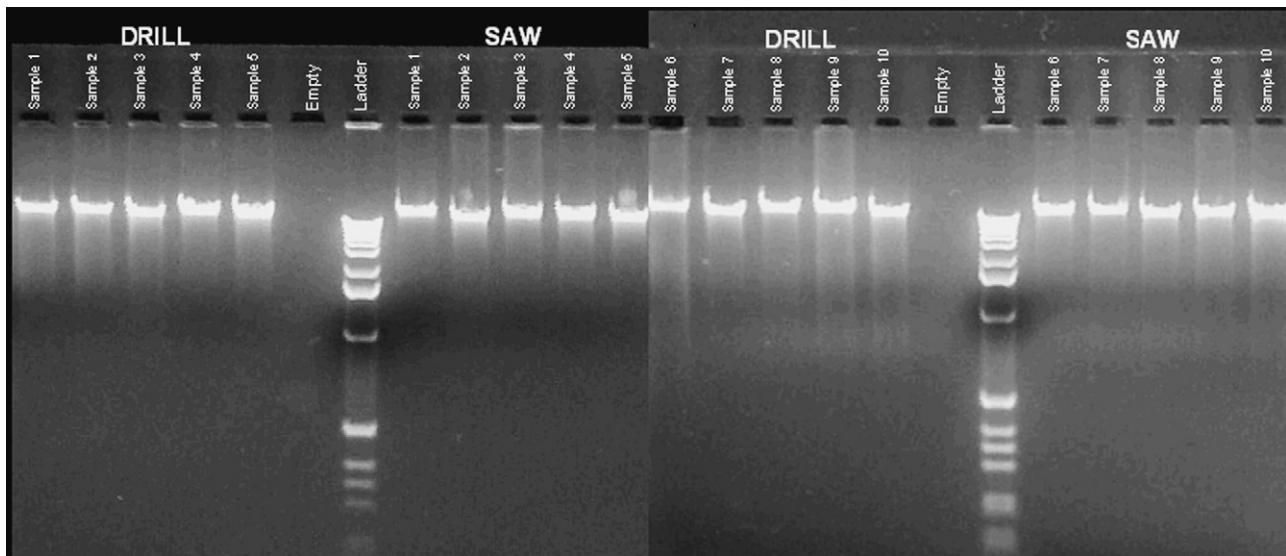


Fig. 1. Assessment of DNA quality on agarose gel. Agarose gel comparing DNA quality of samples from tissue dissected with the saw and with the drill. A Trackbit 1 kb DNA ladder was used, and DNA samples were prepared and loaded using a 6× loading dye.

reported in the form of a RNA integrity number, or RIN (scale between 1 and 10, with 1 indicating high levels of degradation and 10 indicating highly intact RNA; for more information, see <http://www.chem.agilent.com/temp/rad30D9F/00001064.PDF>). Unlike a RIN value that can be used as an index of RNA quality, no such algorithm exists for measuring the quality of DNA. To assess DNA quality, DNA samples were loaded into agarose gels to detect fragmentation.

Gene expression studies using RNA derived from postmortem human brain are dependent upon high-quality RNA, and RNA quality has been shown to be a potentially major confounding variable in studies analyzing gene expression (Lipska et al., 2006; Schroeder et al., 2006). RNA integrity can be compromised by numerous factors, including agonal state, postmortem interval, storage temperature and time, the manner and temperature in which the tissue or extracted RNA is handled, and/or the presence of RNases (Schoor et al., 2003; Fleige and Pfaffl, 2005; Lipska et al., 2006). To date, little attention has been directed to the effects of dissection technique on RNA or DNA quality.

Although the use of a small handsaw has served as a traditional, effective technique for human brain dissection, it is time-consuming and often anatomically imprecise. The high-speed dental drill is more anatomically precise when dissecting irregularly shaped regions of interest, like cortical grey matter. Structures deep in the brain or small in size are also more easily dissected using the dental drill, because with the handsaw one must first cut through intervening tissue to reach these structures. Additionally, we estimate that the drill allows the dissection to be completed approximately four times faster than when the handsaw is used. Finally, we have observed that tissue fracturing is more likely to occur when using the handsaw than with the dental drill.

Our results indicate that RNA and DNA are not significantly affected by using either a hand-held saw or the high-speed dental drill and therefore support the use of an electric dental drill as an

efficient and anatomically precise means of human brain dissection without compromising tissue quality.

5. Conclusions

The results were consistent with our prediction that these two dissection techniques are equivalent with respect to RNA or DNA quality. Therefore, these results support the use of an electric dental drill as an efficient and anatomically precise means of human brain dissection without compromising tissue quality.

Acknowledgements

This research was supported in part by NIMH-COR Grant # MH-16573. This work was supported by the Intramural Program of the National Institutes of Health–National Institute of Mental Health.

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