SOFTWARE TOOL ARTICLE

REVISED A tool for reproducible research: From data analysis (in R) to a typeset laboratory notebook (as .pdf) using the text editor Emacs with the 'mp' package [version 2; peer review: 2 not approved]

Growth and motility of a melanoma cell line are inhibited in the presence of *beta*-hydroxybutyrate.

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Abstract

Software

Much scientific research makes use of commonly available 'office' software. While numerous more fully-featured open-source alternatives exist, the integration of diverse tools and platforms which their use often entails can be challenging. The **mp** package for Emacs aims to bring together a number of these elements with the goal of simplifying the process of converting an .R file, as used for data analysis, to a nicely formatted .pdf which includes the complete description of the methods and interpretation. We discuss the rationale for development of the package and illustrate its applications and options with a series of experiments from our laboratory.

Experimental work

We demonstrate the inhibitory effects of the ketone body *beta*-hydroxybutyrate (BHB) on the growth and motility of a cancer cell line. BHB is produced endogenously; levels may be increased in certain medial conditions e.g. diabetic ketoacidosis. They may also be raised voluntarily e.g. by adopting the ketogenic diet.

BHB is known to inhibit the growth of other neoplastic cell lines. However the finding that it can do so in a cell line selected for their propensity to metastasize to the brain is novel. Given the challenges in treating patients with melanoma metastatic to brain, this work strengthens the rationale for investigating the ketogenic diet as a potential adjunct to treatment in such cases.

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Any reports and responses or comments on the article can be found at the end of the article.

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REVISED Amendments from Version 1

We have re-structured the article and added some new sections and references in order to address the issues raised by the referees.

See referee reports

Introduction

Motivation

One of the primary goals of any experimental research is to produce a nicely typeset document which explains the methods and results. This should be sufficient to allow the reader to recreate the work and thus to verify the results (given the correct tools).

In practice, much research is documented by adapting existing ‘office’-type software for this purpose (Microsoft, OpenOffice etc.).

While there is much to be said for the ease of use of these techniques, they are not ideally suited to the purpose. In particular, those that employ a ‘point-and-click’ graphical user interface (GUI) make it impossible to recreate these steps (mouse movements and clicks). The options for generating graphs and analysing data are typically limited and often require the use of separate ‘third-party’ software for these steps (e.g. SPSS, GraphPad Prism). This again makes the reproduction of results a challenge.

There are many free and open source alternatives which are designed with the needs of the laboratory researcher in mind. Ease of use appears to be the principle reason for their lack of widespread use. The **mp** (for ‘*make-pdf*’) package came about as an attempt to bring elements from a number of these diverse sources together. Data analysis is performed using R¹. Typesetting is performed using LaTeX, which has become the industry standard for scientific publications^{2,3}. **mp** was also motivated by the repetitive nature of much laboratory research. Successive experiments often differ little in method and the analysis often uses the same techniques with new data each time.

Emacs is the text editor which brings these methods together. Emacs itself has been criticized for lack of ease of use, although if used purely as a text/file editor, as in the examples here, it remains quite simple⁴.

The examples do require some familiarity with R. The transition from familiar GUI-style data-analysis to terminal-based output may also appear daunting at first. For those considering taking the plunge we hope that these simple examples will help to illustrate how easy the language can be to use. As a long-term investment, we feel that the time taken to become familiar with these methods is likely to be more than compensated by subsequent improvements in the speed and simplicity of work-flow.

What does mp do

mp is a collection of functions and variables which makes and displays a .pdf from ‘the materials available’. It aims to do so ‘at the touch of a button’. It works primarily with the following file types:

1. .R
2. .Rnw or .org
3. .tex
4. .el

The final .pdf is generated with latexmk⁵. **mp** supports the use of indexes, glossaries, nomenclatures and bibliographies (e.g. with a separate .bib file)⁶⁻⁸.

The output from the whole process is shown in a new window; once complete, errors, warnings and success are highlighted; this typically makes correcting errors more straightforward. Once the .pdf has been generated, it is opened with a viewer (by default ‘evince’⁹).

The red arrow in **Figure 1** shows the default route taken through these file types. The function `mp-mp` can be called on any of the file types above, or on a directory containing such files. Thus it may be used solely, for example, in converting .tex to .pdf.

When the process is repeated, prior files are as over-written. Thankfully, Emacs will, by default, save files automatically when modified, although clearly caution is still required.

The most important variable is the intermediary (or ‘go-between’) file and the step which follows. This intermediary is either a no-web (.Rnw) or an .org file^{10,11}.

.Rfiles. Typically, it is easier to perform data analysis using an .R file directly vs. a more cumbersome intermediary .Rnw or .org.

The .R file is broken up into ‘chunks’ of code, which (by default) correspond to sections in a corresponding LaTeX document (to be generated). These are separated by headers: `## ---- chunkName`. This follows the convention introduced by **knitr** for naming chunks.

No other ‘markup’ is employed when processing the .R file. If .org is used as the intermediary, there is an option to convert LaTeX math mode in the .R comments to inline math in LaTeX (as shown later in example 4).

A typical use case would thus involve writing R code (in a .R file), using **mp** to generate an ‘intermediary’ .Rnw file then ‘fleshing out’ the latter to include additional explanatory text:

1. Create a new directory to hold all the files below.
2. Draft the code for analysis in .R.
Arbitrary ‘placeholder’ data may be used at this stage.
3. Run **mp** to create a ‘draft’ or ‘skeleton’ .pdf from .R.

4. Use the intermediary `.Rnw` file generated to add an experimental protocol.
5. Complete the experiment.
6. Update the `.R` or intermediary file generated with the results and conclusions.
7. Run **mp** again on the intermediary `.Rnw` file to generate the final `.pdf`.

.Rnw files. Another approach is to write the `.Rnw` file directly. R code can be integrated into the document; alternatively, an `.R` file with the same name may be used for the code chunks; **mp** will try to match the chunks in that file to the sections in the `.Rnw` document. New sections are added, as necessary, while preserving the a common order between the documents where possible.

Entwiners

The intermediary file is then passed on to one of the tools below. Following the existing trend to name these instruments after

processes involved in fabric making, we refer to them collectively as ‘*entwiners*’.

- **knitr** (‘knitter’) - the default for **mp**.
- **Sweave** (‘S-weave’).
- Emacs' own **Org** mode (herein **org-mode**).

mp uses templates (particularly for the preamble) when generating intermediary files; there is one for each entwiner. These contain defaults that are based on the authors’ own work-flow. For example, the LaTeX package **siunitx** is included to allow for the correct display of scientific units¹². Our ‘hello world’ example (Listing 2 below), while too simple to make use of the additional packages loaded, does show how multiple options are set in LaTeX and R (including **knitr**).

.el files. **mp** can also generate documentation for an elisp package which is contained in one file. This is done using `.org` as the intermediary. This feature was added to emulate the nicely typeset package documentation that is standard in R and LaTeX. As an example,

Figure 1. Flow diagram showing file types and converters in the **mp** package. The red arrow shows the default flow path.

the manual for the **mp** package itself was generated this way and is given as [supplementary material](#).

Control flow

A more detailed diagram, which also shows the customizable variables, is available as [Supplementary material](#) (mpFlow.pdf). Some familiarity with setting ‘customizable variables’ is required when moving beyond the default settings for **mp**. For more experienced users, knowledge of these functions and variables allows for highly granular control, if desired.

Why elisp?

mp depends on an array of tools. Some are command-line, some use R and some are Emacs packages^{5,11,13–15}. We settled on elisp, the native language of Emacs as it allows for easy integration of these diverse methods ‘under one roof’¹⁶. There is a good deal of ‘text manipulation’ involved; as an extensible text-editor, Emacs is particularly well suited to this task.

Elisp supports *asynchronous* processing. That is, the Emacs terminal is not ‘frozen’ during the process of .pdf generation and thus an R session or text editing can continue uninterrupted.

Lisp itself, while no longer popular, is arguably comparable in efficiency and speed to any of the widely used programming languages. It can be interpreted, allowing for rapid development; and compiled, allowing for improvements in speed when required. While the syntax is often said to be ‘off-putting’ initially, it may also be described as ‘expressive’, allowing for the concise and efficient representation of problems. *Any* language with the ability to write to and read from files and send text to the command line could have been used for the purpose. Thus the methods could have been implemented in R or LaTeX, although perhaps in a more lengthy and less readable form. Doing so would also lose some of the tight integration with Emacs which was a goal of the package.

Related tools

Methods used by mp

Converting from an intermediary to a .tex file is performed by one of the entwiners shown in [Table 1](#).

Sweave. This is the oldest and best-supported of these converters¹³. It is *the only* such method supported by R-core. It continues to be used as a standard tool for R package developers writing vignettes.

It does suffer from a number of limitations relative to its counterparts. In particular, the displayed code ‘as-is’ has little formatting and no color. Only one figure per ‘chunk’ is supported. This may be overcome by reading/writing files in R directly, although this can be tricky to implement.

Knitr. This has superseded Sweave for most practical purposes¹⁴. The code is much easier to read. It also allows for multiple figures per chunk, with their own captions. It allows the chunks to be kept in a separate source file - as opposed to requiring them to be part of the .Rnw file. There are more options for the display of terminal output, including handling of error messages. Like **Sweave**, it can be used to build R package vignettes.

org-mode. .org files are arguably more intuitive to read and edit than .tex, particularly for users new to the latter. Tables are simpler to read, create and modify. The use of ‘collapsible’ section headers makes it easy to see the structure of a document at-a-glance before expanding one section for further editing^{17,18}.

While org-mode loses the attractive code printing of **knitr**, some worthy alternatives are provided by the LaTeX package **listings**¹⁹. In **mp**, the default settings adopted for **listings** are modeled after the **knitr** defaults (although admittedly not quite as attractive).

These include the option to include LaTeX maths markup in the code commentary, for example to display equations.

Like **Sweave**, org-mode chunks suffer from the drawback that multiple figures per chunk are not supported by default.

By contrast with the other entwiners, Org-mode allows for conversion/export to multiple file types. By default, **mp** converts .org to .tex but alternatives are straightforward, such as to .html or to .MARKDOWN.

Allied approaches

Converting .org to .Rnw is possible using the **ravel** package for Emacs²⁰. This is also possible with the **pander** package for R, which integrates R with the Haskell library **pandoc**^{21,22}. It is broader in scope than **ravel** and aims to convert a wide range of file types. Both could serve as alternatives to any of the entwiners above or be used in conjunction with them. We have chosen to stick with the three above as these appear better established.

Table 1. Choice of mp-entwiner when converting from .R to .tex.

Method	Advantages	Disadvantages
knitr	Nice code formatting	Settings maybe incompatible with other loaded LaTeX packages
Sweave	Supported by R-core .tex files easy to read	More limited options than knitr Only one figure per chunk
Org	Tables easy to write Markup is simpler than LaTeX Can include code from other languages Use math in code comments Export to HTML	Formatting not as ‘nice’ as knitr Only one figure per chunk

Closer integration between R and LaTeX is possible using the LaTeX packages **knitr** and **spaper**; although not yet part of the Comprehensive TeX Archive Network (CTAN), they are readily available on github^{23,24}. The author, Dr F Harrell, also provides some very useful .Rnw templates for use in statistical reporting; we encourage the reader to explore these methods at <https://github.com/harrelfe/rlatex>. **mp**, by contrast, takes the approach of storing its templates as customizable variables within Emacs. This has the potential advantage of having them ‘close to hand’ when working in Emacs, easy customization, type-checking and persistence across Emacs sessions.

Alternatives to mp

There are many other good tools available which aim to bridge the gap between .R and .pdf, although to our knowledge, **mp** is the one which allows the user to generate one file type from the other with a single keystroke. We suggest that having more options available for the task is by no means a bad thing; doubtless some of these methods will be more appealing depending on the users background or the task at hand.

RStudio. This integrated development environment (IDE) is the leading alternative to Emacs for working with R and generating .pdfs²⁵. It is probably the best choice for those new to combining R and LaTeX. It has a ‘friendlier’ GUI than Emacs and the menus arguably simplify access to functions. It has better integration with **Rmarkdown** (see below)²⁶. XeLaTeX is supported, although at the time of writing LuaLaTeX does not appear to be (in contrast to **mp**).

Having used RStudio on a daily basis for two years, our first author ultimately found Emacs to be preferable, primarily on account of the gain in speed when editing text/code and also due to the ability to customize and improve the environment as required for specific tasks. With RStudio, there is also the major drawback when compiling .pdfs that the application *pauses* with no output until the process is complete. It is also typically more ‘memory hungry’ than Emacs. If running multiple R sessions, a new copy of the application needs to be opened for each. These limitations are minor for small files and data sets but can become a major inconvenience with more complex tasks. Finally, RStudio requires a license for commercial use, whereas all the elements in **mp** are freely available.

Rmarkdown. This R package is another way of feeding text and code to **knitr**. It combines the attractive features of the latter with a simplicity of style similar to org-mode. It too allows for export to multiple file types, including .html and Microsoft .doc. The process generates an additional .md file which is then processed by **pandoc**. Due to the additional dependencies introduced, we have not sought to include these methods in **mp**.

knitr. This entwiner already integrates with some GUI-style .tex editors, particularly LyX. The latter is part of ‘Scientific Workplace’ (SW), which, like **mp**, tries to make life easier for the laboratory researcher by providing a simplified work space. In the case of SW, a GUI is preferred to directly editing files²⁷.

Minted. This is an alternative to the **listings** package for typesetting code with LaTeX²⁸. While admittedly often more attractive for code

display, it requires an external python dependency. Also, for code chunks which span more than one page, automating background coloring is currently challenging.

Experimental work: BHB, cell growth and migration

Beta-hydroxybutyrate is a source of energy produced by the liver when the body is in ketosis, i.e. when the availability of glucose/sugars as a source of fuel is limited.

Increasing ketones in the blood lead to higher rates of fatty acid oxidation and an increase in the production of acetyl-CoA. When the amount of acetyl-CoA exceeds the capacity of the tricarboxylic acid cycle to utilize it, there is an increase in the production of the ketone bodies (BHB and acetoacetate (AcAc)).

One of the hallmarks of cancer is the dysregulation of metabolism. Cancer cells are particularly dependent on glucose as an energy source whereas normal tissue can readily adapt to using ketone bodies as an alternative. This is in part due to genetic and mitochondrial defects in cancer cells^{29–35}.

Thus, a number of treatments involving the modification of diet to stimulate ketone production have been suggested: the ketogenic diet, caloric restriction and intermittent fasting. These strategies have been studied in various *in vivo* models of glioma, a malignant brain tumor. They have demonstrated increased survival as well as anti-tumor effects³⁶.

The ability of BHB to inhibit cancer cell growth and migration has long been recognized^{37–39}. The work from Magee *et al.*, from 1979, also features the B16 melanoma cell line. These investigators demonstrated a reduction in the number of lung metastases following an injection of cells to the tail vein of mice, in those receiving a diet of just fats and water vs. sucrose and water.

This phenomenon is of particular interest to our laboratory as we have demonstrated that the ketogenic diet (whereby energy requirements are met almost exclusively with fat) enhances the response of glioma to radiation and chemotherapy in a mouse model⁴⁰.

We sought to determine whether the same phenomenon would be observed with other cancer types which are commonly metastatic to the brain; in particular melanoma.

Methods

Software implementation

The software may be obtained via e.g. `git clone https://www.github.com/dardisco/mp`

The following should then be placed in your `init.el` file:

```
(add-to-list 'load-path "/path/to/mp")
(require 'mp)
```

mp should then be available once Emacs starts. It is a ‘minor mode’ for Emacs; the sole keybinding invokes the function `mp-mp` with `Ctrl-Alt-[` (usually found above the ‘Return’ key; in Emacs parlance

this is also known as C-M-). `mp-mp` is a gateway to all of the package functions; these can also be run individually/‘interactively’ as required (using `execute-extended-command`).

`mp-mp` prompts for a file name; if none is supplied, it will look first at the current buffer. If this is not an `.R`, `.Rnw`, `.org` `.tex` or `.el` file it will select the appropriate file from the default-directory as that which has most recently modified. Thereafter it will search up the directory tree if no such file is found.

Instead of a file name, the single character ‘p’ may be given to display the appropriate `.pdf` associated with the current file or directory.

Operation

System requirements: this should work with any recent version of Emacs, which is platform independent (i.e. works on Windows, Linux, Mac-OS). Version ≥ 24.4 is recommended to allow for automated export of `.org` to `.tex`.

To export to HTML, the elisp package **htmlize** is required¹⁵.

A recent installation of R (>3.0) and TeX (2013 and on) is also assumed. We used TeX Live 2013 for these examples.

No support for caching is provided, although with short documents similar in scale to the examples below this should not result in much loss of performance. The time to compile is <10 secs with an Intel i5-2430M processor for all of the examples given. When speed is an issue, we suggest temporarily changing your `PATH` to allow the relevant R and LaTeX binaries to run from a temporary RAM drive.

‘Hello world’ with mp. We begin with the simplest use case. We create the directory `hello` and place the contents of Listing 1 into `hello.R`.

Listing 1: The file `hello.R`

```
## ---- Print hello
print("Hello world")
## ---- Here are two graphs
## here's a comment
plot(1:10, 10:100)
## this second graph should appear
## below the first
plot(1:10, sqrt(1:10))
```

The final product, `hello.pdf` is shown in Figure 2.

The intermediary file `hello.Rnw` will be left open in Emacs for further modification and this is shown in Listing 2.

Listing 2: The file `hello.Rnw`

```
%
\documentclass{article}
%
% modified from default setup for knitr
%
\usepackage[]{graphicx}
\usepackage[]{color}
\usepackage{framed}
% recommended with 'knitr'
\usepackage{alltt}
\usepackage{mathtools}
\usepackage[sc]{mathpazo}
\usepackage{geometry}
\geometry{verbose, tmargin=2.5cm, bmargin
=2.5cm,
lmargin=2.5cm, rmargin=2.5cm}
\setcounter{secnumdepth}{2}
\setcounter{tocdepth}{2}
\usepackage{url}
\usepackage{hyperref}
\hypersetup{unicode=true, pdfusetitle}
\hypersetup{bookmarks=true,
bookmarksnumbered=true}
\hypersetup{bookmarksopen=true,
bookmarksopenlevel=2}
\hypersetup{breaklinks=false, pdfborder={0 0
1}}
\hypersetup{backref=false}
\hypersetup{colorlinks=true}
\definecolor{myDarkBlue}{rgb}{0, 0, 0.5}
\hypersetup{linkcolor=myDarkBlue}
\hypersetup{pdfstartview={XYZ null null 1}}
%
% Palatino family
\usepackage[T1]{fontenc}
\usepackage{mathpazo}
\linespread{1.05}
\usepackage[scaled]{helvet}
%
% other useful additions
%
%% for rerunfilecheck:
% no need to rerun to get outlines right
\usepackage{bookmark}
%% for nice tables
\usepackage{booktabs}
%% for e.g. \formatdate
\usepackage{datetime}
%% for SI units
\usepackage{siunitx}
%% for chemical symbols
\usepackage[version=3]{mhchem}
```

```

%% to use forced 'here'
%% e.g. \begin{figure}[H]
\usepackage{float}
%% for large numbers of floats
\usepackage{morefloats}
%% to keep floats in same section
\usepackage[section]{placeins}
%% for tables > 1 page
\usepackage{longtable}
%
%%-----
%
\begin{document}
%
% knitr chunks
<<setup, include=FALSE>>=
library(knitr)
### Set global chunk options
opts_chunk$set(
  eval=TRUE,
  ## text results
  echo=TRUE,
  results=c('markup', 'asis', 'hold', 'hide')
    [1],
  collapse=FALSE,
  warning=TRUE, message=TRUE, error=TRUE,
  split=FALSE, include=TRUE, strip.white=TRUE,
  ## code decoration
  tidy=FALSE, prompt=FALSE, comment='##',
  highlight=TRUE, size='normalsize',
  background=c('#F7F7F7', colors()[479], c
    (0.1, 0.2, 0.3))[1],
  ## cache
  cache=FALSE,
  ## plots
  fig.path=c('figure', 'figure/minimal-'),
  fig.keep=c('high', 'none', 'all', 'first', 'last')[1],
  fig.align=c('center', 'left', 'right', 'default')[1],
  fig.show=c('hold', 'asis', 'animate', 'hide')[1],
  dev=c('pdf', 'png', 'tikz')[1],
  fig.width=7, fig.height=7, #inches
  fig.env=c('figure', 'marginfigure')[1],
  fig.pos=c('', 'h', 't', 'b', 'p', 'H')[1])
### Set R options
options(formatR.arrow=TRUE, width=60)
knit_hooks$set(inline = function(x) {
  ## if (is.numeric(x)) return(knitr::format
    _sci(x, 'latex'))
  highr::hi_latex(x)
})
## uncomment below to change theme
## knit_theme$get()
## opts_knit$set(out.format='latex')
## thml <- knit_theme$get('acid')
## knit_theme$set(thml)

```

```

@
% knitr read chunks
<<readChunks, include=FALSE>>=
read_chunk('hello.R')
@
\title{hello}
\author{Chris Dardis}

\maketitle
% page numbers appear top-right
\pagestyle{headings}
\tableofcontents

\subsection{Print hello}

<<Print hello>>=
@

\subsection{Here are two graphs}

<<Here are two graphs>>=
@
%
%% \bibliographystyle{plain}
%% \bibliography{hello}
\end{document}

```

Experimental work

The purpose of the current work was to demonstrate an inhibitory effect of BHB on the growth and migration of a melanoma cell line *in vitro*. Details of the aims, methods, results and conclusions are given for each experiment separately as [Supplementary material](#) (see [Table 2](#)).

These experiments would normally make up just one part of an article, where it would be reasonable to combine all of these as one supplement. We provide the experiments separately for the sake of illustrating features of the **mp** package.

Cells. Melanoma B-16 cells were obtained from American Type Culture Collection (ATCC). To facilitate quantitative measurement of tumor growth, they were modified as described previously⁴¹. The cells were stably transfected with the gene encoding luc2 (luciferase) using the pGL4.51 [luc2/CMV/Neo] vector (Promega Corp, Madison, WI) and FuGENE[®] 6 Transfection Reagent (Roche Applied Science, Indianapolis, IN) following conditions specified by the manufacturer. They were then injected into the right ventricle of a mouse. These animals were sacrificed when bioluminescence was detected in the brain. Cells metastatic to the brain were recovered put into culture. These cells are designated B16-F1-Luc2-BR2.

Effects of BHB on growth. Firstly, it was necessary to determine the optimal starting number of cells to seed a growth curve. If the cells are plated too sparsely, they have a tendency not to grow. Likewise if the concentration is too great to start with, then this will make any difference due to the effect of BHB difficult to see. This is

(2.1)

hello

Chris Dardis

Thursday 7th May, 2015

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0.1 Print hello

```
print("Hello world")  
  
## [1] "Hello world"
```

0.2 Here are two graphs

```
## here's a comment  
plot(1:10, (1:10)^2)  
## this second graph should appear  
## below the first  
plot(1:10, (1:10)^-1, type="b", col="blue")
```

(2.2)

(2.3)

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Figure 2. The file `hello.pdf`.

Table 2. Steps taken in generating examples.

	File	Entwiner	Edit	TeX	Final
1	8k	knitr	.Rnw*	LaTeX	.pdf*
2	16k	knitr	.Rnw*	LaTeX	.pdf*
3	16k2	Sweave	.Rnw*	XeTeX	.pdf*
4	32k	Org	.org*	LaTeX	.pdf*
5	32k2	Org	.org*	LaTeX	.html*
6	sa	knitr	.Rnw*	XeTeX	.pdf*

* = file available as [Supplementary material](#)

complicated further by growing the cells in a circular well - the cells are in closer communication close to the center and more separated close to the edge. One technical report suggested a starting number of 4000 per well in a 12-well plate for most cell lines⁴². In choosing a starting number, we were guided primarily by prior work in our own laboratory.

To determine whether BHB would inhibit cell growth, we chose a concentration of 10 mmol/L, again based primarily on our prior experience. Under normal conditions, serum BHB has an upper reference limit of 0.3–0.5 mmol/L. Tests of BHB concentration (for patients) are available commercially and are commonly used to aid in the diagnosis of diabetic ketoacidosis, where levels are usually at least 2 mmol/L.

Even higher levels are seen in patients adhering to the ketogenic diet. This diet is most commonly employed in the treatment of drug-resistant epilepsy, where serum BHB concentration has been shown to correlate with the degree of seizure control⁴³. In this study of 74 children, median levels were 8–10 mmol/L.

We chose a concentration of 10 mmol/L in order to be certain that an effect was present, before considering whether lower levels would be similarly efficacious.

Effects of BHB on migration. The scratch assay is a simple and widely used test to assess cell migration. Details of a typical protocol for the technique are available⁴⁴. Using the same cell line, we sought to illustrate impaired migration in the presence of BHB.

Results

Each experiment is contained in a separate file, shown in [Table 2](#). These are all available as [Supplementary material](#) and we encourage the reader to have these available for reference. Each experiment is typeset in a different way to demonstrate some of the options available when doing so with **mp**.

The file stems (i.e. the file name without a suffix) refer to the starting quantity of cells per 12-well plate. 'sa' in the last example stands for 'scratch assay'.

Experiment 1: 8k.pdf

Experiment: Establish number of cells required to establish sustained growth. Start with 8000 cells per 12-well plate. Our first aim was to establish the optimal number of B16-F1-Luc2-BR2 cells with which to seed a growth curve. We wished to establish sustained growth over the period of the experiment, without reaching a ceiling (i.e. maximum density).

Typesetting: default settings. The file 8k.R is given in [Listing 3](#):

Listing 3: The file 8k.R

```
## ---- data
(df1 <- data.frame(Day = rep(0:4, each=3),
                   Well = rep(LETTERS[1:3], 5),
                   Count = c(rep(8e3, 3),
                             193e3, 148e3, 159e3,
                             78e3, 63e3, 79e3,
                             33e3, 56e3, 33e3,
                             13e3, 15e3, 10e3)))

## ---- stdError
stdErr <- function(x) sqrt(var(x)) / sqrt(
  length(x))
library(plyr)
(df2 <- ddply(df1, .(Day),
              function(df)
                c(mean = mean(df$Count),
                  SE = stdErr(df$Count),
                  'mean+SE' = mean(df$Count) +
                    stdErr(df$Count),
                  'mean-SE' = mean(df$Count) -
                    stdErr(df$Count))))

## ---- plot
library(plotrix)
# confidence interval plot
with(df2, plotCI(x=Day, y=mean, ui=mean+SE, li
                 =mean-SE,
                 xlab="Day",
                 ylab="Cell counts (Cells/ ml)",
                 axes=FALSE))
axis(side=1, at=c(0:4))
axis(side=2, at=seq(0, 150e3, by=50e3))
box()
with(df2, lines(Day, mean))
```

With the above file open, we call **mp-mp** to generate the a.pdf; page 1 of the result is shown in part in [Figure 3](#).

As an intermediary step, the file 8k.Rnw is generated. This is an R no-web file, which is essentially a typical .tex file with the additional code chunks inserted¹⁰. (The term 'web' is not in reference to the world-wide web but to distinguish it from a contemporaneous approach to literate programming known as 'web'.)

8k2

Chris Dardis

Monday 6th April, 2015

Contents

0.1 data	1
0.2 stdError	2
0.3 plot	2

0.1 data

```
df1 <- data.frame(Day = rep(0:4, each=3),
                  Well = rep(LETTERS[1:3], 5),
                  Count = c(rep(8e3, 3),
                             193e3, 148e3, 159e3,
                             78e3, 63e3, 79e3,
                             33e3, 56e3, 33e3,
                             13e3, 15e3, 10e3))

library(xtable)
print(xtable(df1,
             caption="Cells per ml, day 0 - 4",
             align=c("l", rep("c", 3)),
             display=c("d", "d", "s", "fg"),
             label="data"),
      booktabs=TRUE)

## % latex table generated in R 3.0.1 by xtable 1.7-1 package
## % Mon Apr 06 08:59:33 2015
## \begin{table}[ht]
## \centering
## \begin{tabular}{lccc}
## \toprule
## & Day & Well & Count \\
## \midrule
## 1 & 0 & A & 8000 \\
## 2 & 0 & B & 8000 \\
## 3 & 0 & C & 8000 \\
## 4 & 1 & A & 193000 \\
## 5 & 1 & B & 148000 \\
## 6 & 1 & C & 159000 \\
## 7 & 2 & A & 78000 \\
## 8 & 2 & B & 63000 \\
## 9 & 2 & C & 79000
```

Figure 3. Page 1 of the initial 8k.pdf.

We modify this to add details of the experimental protocol, written in LaTeX. The principal modifications are the use of 12pt for typesetting, which we adopt from hereon and the inclusion of some graphics with the LaTeX **subfig** package⁴⁵.

With 8k.Rnw still open, we call mp-mp again to generate the final .pdf.

Experiment 2: 16k.pdf

Experiment: Number of cells required to establish sustained growth. 16000 cells per 12-well plate. Again, we tried to establish the optimal number of B16-F1-Luc2-BR2 cells with which to seed a growth curve. We increased the starting number of cells per well from 8000 to 16000. This appeared to be more promising.

Typesetting: using R to generate .tex output. We again begin with an .R file. This time however, we use R to create LaTeX output, rather than displaying the results of a chunk as R code. The output from R is interpreted as LaTeX directly - rather than as typical terminal output. We use the R **xtable** package for this purpose; there are many good alternatives⁴⁶.

An example of R code generating output which can be interpreted by LaTeX is shown in [Listing 4](#).

Listing 4: From the file 16k.R

```
## ---- Cell counts on Day 0
df1 <- data.frame(Cells_per_ml = c(392e3, 332
  e3, 300e3))
library(xtable)
print(xtable(df1,
  caption="Cell counts on day 0",
  align=c("l", "c"),
  display=c("d", "e"),
  label="tab:ccd0"),
  booktabs=TRUE)
```

Again, the .Rnw file needs some modification. The main change is to set the knitr option results='markup' to results='asis' to allow the results to be interpreted as .tex.

We can also restructure the document to allow the code output (here, in the form of tables or single lines of terminal output) to be mixed with the main text. This ensures there is no needless duplication of data entry, as is the case in the earlier example.

The output is shown as [Supplementary material](#) in 16k.pdf. Most of the materials and methods are unchanged from 8k.pdf.

Experiment 3: 16k2.pdf

Experiment: Demonstrate impaired growth in the presence of BHB. 16000 cells per 12-well plate. We sought to determine whether the growth of B16-F1-Luc2-BR2 cells would be impaired in the presence of BHB at a concentration of 10 mmol/L.

Due to the large standard error on day 4, no difference could be demonstrated via a t-test. However comparing linear models with and without treatment *did* show a significant effect (analysis of variance (ANOVA) $p = 0.048$).

Typesetting: using Sweave and XeTeX. Here, we change the variable mp-entwiner to Sweave. We also change mp-latex to XeLaTeX to allow the use of an OpenType font (by default this is 'Lix Libertine'). While certain OpenType fonts are available as packages for LaTeX, XeTeX allows for a greater range and greater flexibility.

As in example 2, the output from each chunk is again typically interpreted as .tex (rather than displayed as R code) by including results=tex in mp-Sweave-opts.

Some modifications to the .Rnw file generated follow. There is one chunk where we prefer the output to retain typical R code formatting. Also we need to change the variable mp-Sweave-opts to fig=TRUE for the chunk which produces a plot.

This example also shows the value of LaTeX for typesetting equations - calculation of correct masses are nicely displayed and easy to follow. This is shown in [Figure 4](#).

Experiment 4: 32k.pdf

Experiment: Demonstrate impaired growth in the presence of BHB. 32000 cells per 12-well plate. As the preceding experiment was inconclusive, we tried the same technique again, this time using a higher number of cells to start with.

Unfortunately, no observations were taken on day 3 of the experiment. This meant that both the t-test on day 4 and the ANOVA comparing linear models were not significant, although the latter did come close ($p = 0.07$).

Typesetting: using Org. Changing mp-entwiner to Org allows us to use an .org file as the intermediary step.

The output from this process is given as 32k.pdf. Again, some minor modifications to the intermediary file are required. In this case we need to change the headers for the chunk which produces a plot to specify the output of graphics (as opposed to text) and to specify an external file for the graphical output.

We also change some of the results to latex and pp (pretty print) to give examples of different styles of output. 32k.pdf also features a printout of help for an R function.

Experiment 5: 32k2.pdf

Experiment: Demonstrate impaired growth in the presence of BHB. 32000 cells per 12-well plate. We repeated the preceding experiment and this time there was a conclusive difference between the cell counts in the those grown with BHB vs. controls.

$$1 \text{ mol} = 126 \text{ g} \quad (1)$$

$$10 \text{ mmol} = 1.26 \text{ g} \quad (2)$$

$$10 \text{ mmol/L} = 1.26 \text{ g/L} \quad (3)$$

$$= 0.00126 \text{ g/mL} \quad (4)$$

Thus 0.00126 g in 10 μ L added to 1 mL gives:

$$0.00126 \text{ g per } 1 \text{ mL} = 10 \text{ mmol/L} \quad (5)$$

Figure 4. Typeset equations, from 16k2.pdf.

Typesetting: using Org to generate HTML output. This example shows how the .org intermediary can be converted to .html as an alternative to .pdf. Converting to HTML means we lose all but the simplest LaTeX commands and so the document needs to be written in a simpler style. While we lose some of the nice typesetting features of LaTeX, this simplicity has its own attraction.

These files are faster to produce and smaller than their .pdf counterparts (1/5 the size in this example). HTML is also likely to be simpler to integrate into an existing website and offers the possibility of almost ‘real-time’ reporting of experimental results.

Experiment 6: sa.pdf

Experiment: Demonstrate impaired cell mobility in the presence of BHB. Here we use a scratch assay⁴⁴. A line is made in the center of the wells on a 12-well plate with confluent cell growth. Images taken at various time points are analyzed with freely available imageJ software. The distance between the cells on either side of the scratch decreases as they migrate back to the center. This corresponds to a decrease in ‘image density’ as measured by imageJ.

Typesetting: emulating a laboratory notebook with XeTeX. The final example shows how a cursive font may be used to mimic a typical lab notebook. It also shows how graphics may be combined in a table to help with clarity of display. We set mp-entwiner to knitr and set mp-latex to XeTeX to allow the use of an unusual font. A sample is shown in Figure 5.

Summary of significant results. As shown in Figure 6, we were able to demonstrate a difference in growth rates with an initial concentration of cells of 32,000 per well and a concentration of BHB of 10mmol/L. This is shown in full in 32k2.html.

We were also able to demonstrate a decrease in cell migration using the scratch assay, as shown in Figure 7, which is taken from sa.pdf.

Discussion Software

The above examples illustrate some of the options available when using mp. While it may not be the tool of choice for every application, it has been a valuable addition to our own laboratory work and we hope this to be the case for others engaged in similar work.

Much scientific research is reported in the form of a summary and it has not been traditional for researchers to provide detailed accounts of the original experiments along with the original observations/results made at the time. We acknowledge that the details of individual experiments, particularly ‘pilot’ studies of an exploratory nature or those which yielded negative results are likely to be of limited interest to a general readership. Including these as supplements appears a reasonable approach.

The classic quote on reproducible research is from Buckheit and Donoho⁴⁷:

An article about computational science in a scientific publication is not the scholarship itself, it is merely advertising of the scholarship. The actual scholarship is the complete software development environment and complete set of instructions which generated the figures.

The same may well be said of biology. Within a traditional print-form journal, this is justifiable as constraints of space naturally limit the length of articles. However, this has been obviated by the option to refer to online-only **Supplementary material**.

Such resources may be valuable for those seeking to reproduce particular steps in the research. They may also be of interest to those working on closely related problems, in particular by saving time on preparatory work. Providing original ‘raw’ data may also allow results to be combined with subsequent experiments. Furthermore, novel techniques, perhaps not developed at the time of writing, may later be applied by other researchers, perhaps leading to new or deeper insights.

Null hypothesis: mock less than BHB.
Density (percentage of starting value) by treatment."),
label="tab:tt")

	time	p_value
1	0	0.07
2	3	0.04
3	6	0.03
4	20	0.03

Table 3: Welch Two Sample t-test. 1-sided. Null hypothesis: mock less than BHB. Density (percentage of starting value) by treatment.

5.4 Standard error

For plotting, we first calculate the standard error at each time point:

$$SE_x = \frac{s}{\sqrt{n}} \quad (3)$$

where s is the sample standard deviation ($s = \sqrt{\text{variance}}$).

```
stdErr <- function(x) sqrt(var(x, na.rm=TRUE)) / sqrt(length(x))
```

Figure 5. Cursive script in sa.pdf.

Figure 6. Effect of BHB (10 mmol/L) on the growth of B16-F1-Luc2-BR2 cells. Taken from 32k2.html (Supplementary material).

Figure 7. Effect of BHB (10 mmol/L) on the migration of B16-F1-Luc2-BR2 cells. Taken from sa.pdf (Supplementary material).

Experimental work

The inhibitory effects of BHB on the growth of neoplastic cells has now been observed in a number of cells lines in our laboratory, as well as those of other investigators³⁷.

Impairment of growth of neoplastic cells due to ketones has been suggested to be in part due to the polyacetylation of histones⁴⁸. Ketone bodies also impair glycolysis, on which neoplastic cells are more heavily dependent. This occurs in part through inhibition of the activity of phosphofructokinase and hexokinase^{49,50}.

The finding that inhibition of growth occurs in a cell line selected for their propensity to metastasize to the brain is novel. Brain metastases are a significant contributor to morbidity and mortality

in patients with cancer⁵¹. We feel that this approach deserves further investigation. In particular, it would be of interest to determine whether the same phenomenon would be observed in breast and lung cancer cell lines with a propensity to metastasize to the brain. It would also be interesting to determine whether the inhibitory effects of BHB on cancer cells are evident *in vivo* when ketones are given as a dietary supplement with a relatively normal diet - as opposed to the ketogenic diet, which may be challenging to adopt for patients affected by cancer.

Conclusions

Software

The **mp** package appears to be a useful addition to the tools available for facilitating reproducible research. Future develop-

ment will likely include increased support for the LaTeX biblatex package. Collaboration and suggestions for improvement are welcome. Please try to address these to the development site on github if possible.

Experimental work

We have demonstrated here that BHB is inhibitory to the growth and migration of B16 B16-F1-Luc2-BR2 melanoma cells, which were selected for their propensity to form brain metastases, at a concentration of 10 mmol/L.

Data availability

The data sets for all experiments are available as part of the [Supplementary material](#).

One of the primary motivations for developing this package was to facilitate sharing of data sets such as these.

Software availability

Source code

<https://www.github.com/dardisco/mp>

Archived source code as at the time of publication

<http://dx.doi.org/10.5281/zenodo.2118345>

Software license

GPL >= 3

Author contributions

CD - carried out the experiments, wrote the software, performed data analysis and drafted the manuscript.

ECW - designed the protocols for the experiments detailed and carried out the scratch assay experiment.

ACS - supervised the experimental protocols and interpreted the results.

All authors revised and approved the final manuscript.

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

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Supplementary material

A detailed flow diagram for the package is given in `mpFlow.pdf`.

The experiments above are shown in [Table 3](#). The steps taken in generating these files are shown in [Table 2](#).

The intermediary files for the examples are presented as `.html` for ease of display, i.e. `.Rnw.html` or `.org.html`.

This package is 'self-documenting'. `mp.pdf` is the manual for the package, which was generated with `mp-el-tex`. The option `INCLUDESOURCE` is set to `t`, so that the source code is shown for all functions. The intermediary file is `.org` which allows package documentation to be nicely displayed in list or table format using TeX, as appropriate.

Table 3. Experiments.

	Intermediary	Output
1	<code>8k.Rnw</code>	<code>8k.pdf</code>
2	<code>16k.Rnw</code>	<code>16k.pdf</code>
3	<code>16k2.Rnw</code>	<code>16k2.pdf</code>
4	<code>32k.org</code>	<code>32k.pdf</code>
5	<code>32k2.org</code>	<code>32k2.html</code>
6	<code>sa.Rnw</code>	<code>sa.pdf</code>

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Frank Harrell

Department of Biostatistics, Vanderbilt University, Nashville, USA

The authors have significantly improved the article but I regret that I cannot approve an article that implies that analysts should learn Sweave when it is now completely obsolete. In addition, the article fails to take into account the Emacs key bindings were added as a simple option to RStudio in Feb. 2016. So most of the authors' point about Emacs being more efficient, while true in the past, is no longer true. The Emacs learning curve will not be acceptable to 9/10 of users, with RStudio around. RStudio now also has implemented outline mode for very simple navigation of long documents by LaTeX or markdown sections, subsections, chunk names, etc.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Version 1

Reviewer Report 23 November 2015

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Frank Harrell

Department of Biostatistics, Vanderbilt University, Nashville, USA

I cannot approve this article in its current form. The authors may be able to undertake a major revision that makes the paper acceptable.

I realize that this is a subjective opinion, but I just cannot agree with the software implementation chosen by the authors, for the following reasons:

1. Even though I've been using Emacs for more than 20 years and am an advocate of it for the user productivity it brings, I cannot agree that Emacs is the best choice of platform for this application. I say that primarily because RStudio provides the most elegant integrated development environment for R and it is elegantly tied to LaTeX and Markdown, two engines that the authors rightfully advocate.

2. The idea of hiding various complexities from researchers is a noble one, but this can be accomplished in more flexible ways using LaTeX packages. For example, a new Github project at <https://github.com/harrelfe/rlatex> developed by the reviewer provides LaTeX packages `spaper` and `knitr` that streamline reproducible LaTeX reports. Another way to accomplish this is to write R functions that output the needed LaTeX preamble.

The authors refer to both Sweave and knitr but knitr has now completely replaced Sweave. In addition, knitr provides an elegant way to specify figure size and captions.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 08 Mar 2016

Chris Dardis, Barrow Neurological Institute, Phoenix, USA

Apologies for the delay in responding. Thank you for this review; we appreciate the points you raise and feel the article has been improved in addressing these.

1) I (first author) began with RStudio and used the platform for at least 2 years. Ultimately, I moved entirely to Emacs as I found it to be more productive. While not a fully fledged 'integrated development environment', I found this to be more than compensated for by speed and simplicity, particularly in regards to text editing. Most of my work takes place within R; I did feel that integration with LaTeX within Emacs left something to be desired; hence the current package, which allows me to compile documents without leaving Emacs or 'freezing' my R session/ text editing while waiting for a document to compile.

I do acknowledge that RStudio may be a more suitable choice for a particular user. The article has been modified to reflect this; for those who are new to EMACS *and* R/LaTeX integration, I agree that RStudio is likely to be the best initial choice.

We are not recommending 'mp' as the 'best' way to achieve this integration but rather as a viable alternative to RStudio. Surely having a greater range of tools available can do no

harm?

2) I did consider developing a package in LaTeX or R for this end. I was not aware of the tools you yourself have created and am grateful to you for bringing them to my attention; I have referenced these in the main paper. I discuss their merits in the new 'Allied approaches' section in the paper. As far as elisp goes, I think it is well suited for the task at hand; not to say that everything could not be done with R or LaTeX. In the case of the former, it's facilities for processing text/ strings are not, in my view, as simple or as developed as those available in elisp. In the case of the latter, I find development in a non-interpreted language to be slower and more challenging.

"knitr has now completely replaced Sweave" - I disagree; while this may be the case for most practical purposes, Sweave retains the support of R-core and I am not aware of plans for knitr to receive such support. Given the amount of 'legacy' documents generated using Sweave, it is hard to see this changing; thus it will continue to remain important, if increasingly for historical reasons. I agree that knitr should be recommended for most tasks. Sweave does have the advantage of producing .tex files which are simpler to read; it is also a little faster.

"knitr provides an elegant way to specify figure size and captions" - agreed; which I have highlighted this in the main manuscript.

Competing Interests: No competing interests were disclosed.

Reviewer Report 10 September 2015

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Eric Schulte

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The report is insufficiently well written for me to evaluate the importance of the suggested tool.

The current version of this article is unclear as to (1) the functionality provided by mp, (2) the intended usage model of mp. It also (3) conflates an overly-detailed example with the main paper.

1. Is mp merely a translator from a .R format to multiple text document formats? Does mp perform any processing of the input data? Does mp provide stylistic control of the output from within a particular .R file or only through configuration variables?

2. I don't fully understand the usage model. Is prose written **within** the .R file itself, or in the

automatically generated .org, .Rnw or .tex files? If the former what markup syntax does mp provide? If the later does subsequent evaluations of mp overwrite the manually edited intermediate file? These questions should be addressed early on in the article.

3. Finally, an inappropriate emphasis is placed on the “example” experiment. All portions of the example experiment should be typeset to indicate that they are example and their technical content is **not** part of this publication and has not been peer reviewed. As it reads the “results” and “discussion” sections seemed to discuss the content of the example more than the content of the tool.

While it seems very likely that the mp tool is a significant contribution to the practice of reproducible research, I am unable to evaluate its significance from this article.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 08 Mar 2016

Chris Dardis, Barrow Neurological Institute, Phoenix, USA

Apologies for the delay in responding. Thank you for this review; your points are well-taken. I have tried to clarify things by re-structuring the document and adding some new material. We hope that things will appear simpler if you get a chance to try out the package.

1 and 2) Functionality - we have tried to clarify this in the introduction and added a new 'What does mp do?' which should address these points.

3) The experiments **are** part of this publication; they are not present for purely illustrative purposes. The original focus of the manuscript was on the experimental work rather than the software; following discussion with the editorial office at F1000 research, we changed the focus to bring the software to the foreground (which is likely to be of more general interest).

Arguably, the experiments should have been the subject of a separate paper. However, the experiments are short and simple and some 'negative' results are included. Thus we felt it would be more appropriate to include both together (avoiding needless duplication).

Reporting novel methods with new experimental work is reasonably common; it is hard to see how this alone would lead to the conclusion that the material is unworthy of publication.

Understandably, it has been challenging to find reviewers willing to assess the quality of the software **and** of the experiments. It would be reasonable to consider the experimental work 'not peer reviewed' at present if you feel that this lies outside your expertise.

Competing Interests: No competing interests were disclosed.

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