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ACRONYMS

STUFF THAT'S NOT SCIENCE

FLOSS free/libre open source software

GPL GNU General Public License

GNU GNUs Not Unix

PLOS Public Library of Science

OS open science

SCIENCE

GPCRS G-protein coupled receptors

5-HT 5-hydroxytryptamine

CNS central nervous system

BHLH basic helix-loop-helix protein structural motif

VMNS ventral motor neurons

SHH sonic hedgehog

ASCL1 Achaete-scute homolog 1

1 INTRODUCTION

Division III is a year long undergraduate thesis project; for me, it's both articulating and creating I began with neuroscience, and a naive fascination with pathologies of the brain and perception. I have since learned or come to believe that my interests, and what I do with those interests, has an impact on the world – this is both self-interest, for otherwise what's the point? and self-recrimination; my impact on the world is my responsibility, and I need to figure out ways to meld a shattered world view into a practice that has tangible effects on the people and society around me. People are society; if we don't change them, we cannot begin to move forward. Change can come from the top or the bottom, but we write the laws, we execute them, and we – humans – create our societies.

The pathologies of the brain are still intriguing, but so loaded with societal implications that I cannot try to unravel them without also examining the long term effect of *my* research, specifically, but more broadly the impact of cellular-level research etc., on local and global communities.

One tactic I found early in my Hampshire experiences was free/libre software and its sibling in the sciences, open science (more specifically, open access). Both movements acknowledge more than just the functionality of science and technology; they include society in their calculations. While to my current chagrin much of open science does not appear (at first glance, at least) to be founded on the same moral basis as Richard Stallman's free/libre software movement, it is nonetheless science, as a whole, that shares much of that [theoretically] moral spirit. On both practical levels and a deeper moral level, open science seems justifiable and *right* for the same reason free software and free culture feel *right* – they are technologies and creations with a care beyond profit, and give more than a token nod to the users and producers of work. They also often produce amazing and powerful things with an amazingly small amount of money, which makes their farflung rhetoric more believable.

I think, and hope, that open science and its intimate linkage to scientific culture is also an avenue to introduce moral-societal considerations into research. It links research to people.

2 OPEN SCIENCE

2.0.1 *Definitions & interpretations*

"Open science" is a big, vague, nebulous term. First, the short and sweet version:

> "Open means **anyone** can **freely access, use, modify, and share** [content] for **any purpose**."

The "free" in that definition is, of course, free as in freedom, not as in beer. The open science movement is closely affiliated with the free/libre open source software (FLOSS) movement, and as such, there's a large overlap in terminology. Some preemptive clarification:

> "The terminology of and factions within this movement are complex, but, in short, "free software" tends to be associated with the ideology of freedom, "open source" with the openness of the development process, and "libre" with those concerned about confusion from the previous two. FOSS/FLOSS are used as monikers to refer to all of these meanings." (Reagle, 2012)

The open science movement's unifying[1] principle manifests with 3 (or 4, or 6, depending on who you ask) areas of interest:

1. open access - namely, to full-text published papers and research results
2. open data - publishing raw data pre- or concurrently with paper publication
3. open research - everything else, including, but not limited to:
    a) open code distribution - code for analyses, model generation, etc. should be hosted somewhere accessible
    b) open lab notebooks - tracing the entire research process with all dead ends and kinks included

There's also a hundred other subcategories that fall under the umbrella; new systems of distributed, ongoing, or otherwise "open" peer review or community discussion of publications, like the work at PubPeer, billed as an "online journal club". So open science is frustratingly broad and inconsistent, just like any other community. But there are some commonalities in intent and goals, and from that some general open science values can be extrapolated.

2.1 VALUE(S)

I think open science is really interesting as a BIG IDEA, largely because of its ties to the free and open source software movement, and specifically

1 unifying isn't actually the right word here – single shared point? overlap? lots of smaller movements all agreeing on this one

in the realm of community-driven goals. A lot more analytical work has been done on FLOSS sociological values than the narrower and newer niche of OS.

In the FLOSS movement, there's a sharp ideological divide between the free/libre and the open licenses. Advocates of the libre licensing model list many advantages, but their main focus is libre software as a social imperative. User freedom (or their conception of it) is paramount. Open source, on the other hand, was created and remains in an explicit attempt to side-step the social values and ideological connotations of the term "free software", as laid out in the GNU General Public License (GPL) . It instead has a narrow focus on access and production of source code - the 'practical' benefits of distributed production.

Bearing that distinction in mind, trivial as it initially seems, I think the usage of *open* in open science (OS) is a valuable point of entry into the ambivalent moral aspects of open science. Lots of proponents of OS either come from and/or explicitly draw from the F/LOSS movement, but the chosen movement nomenclature is "open". The same disavowal (or maybe just lack of acknowledgment) of social values seen in open source is apparent in a lot of the open science movement. It's not about science helping (or empowering) people, it's about doing "better" science – more reproducible, more reliable, more powerful (disruptive, even!). Open science is defined almost entirely by the *mechanisms*, in the same way open source is *just* focused on production. Even projects that pitch themselves as a place for citizen scientists can be construed as just a way to shift labor from cheap graduate students to even cheaper laypeople.

2.1.1 *"Biohackers"*

Delfanti, in 2013, published "Biohackers: The Politics of Open Science". It lies at the "the intersection of digital cultures, science communication and science and technology studies," a grouping that bears no little similarity to my own interests. Delfanti's book is a delight, and covers in brief detail a number of relevant topics.

Lineages of open (science)

> "The most common narrative about open science tells us that, once upon a time, science was an ethical enterprise: sharing, equality, disinterest and the common good drove the everyday work of scientists. Then evil corporations entered science and changed the rules of the game, patenting life, enclosing the commons, and eventually destroying the willingness to share data, information, and knowledge. But today, so the story goes, we have new tools that together with the old open science spirit an be used to rebel against evil, defeat it and allow scientific knowledge to flow freely again. These tools are open source and open access science, and they can be used to tear down the barriers to the access of scientific knowledge." Delfanti, p. 5

Open science, in almost all narratives I've seen, does exactly this. Drawing on a mystical past where information flowed free ("In the 17th century, journal publication *was* open science!), and in so doing, constructing a seamless narrative where open science is part of every

major discovery since Newton. Or whoever, pick your scientist. The construction of a history lends legitimacy to the moral appeal of the movement.

Public image management

Delfanti profiles 3 projects in his book:

1. The J. Craig Venter Institute and J. Craig Venter himself, representing venture capitalists and open science for monetary and prestige
2. Ilaria Capua, a vetinarian virologist in Italy, who during the 2006 global avian influenza crisis, pushed the WHO through public shaming into changing their data policies to an open access model to better combat the disease
3. "DIYBio", a loose network of homegrown biologists.

Both Venter and Capua used publications in both prestigious journals and in more public forums (*Scientific American*, *The New York Times*) as a way to get the public involved in their work and on their side; public pressure, as conveyed by the media, was influential in both their success stories.

citations here for when I find them

While these are particularly high profile cases, science has an intimate relationship with the media, and both parties use each other as a tool for various goals. The media gets a reputable scientist figure to bolster their claims; the scientist gets a public venue to couch their ideas or goals in objective-but-positive ways.

Corporate uses of open science

Open science, by definition, can be used by anybody for anything. That also means corporate entities can borrow methods, tools, and data released into the public sphere by government-funded organizations

and other organizations, but...beginning analysis

Delfanti does not, however, draw on explicit feminist analysis of race and gender in the spheres he discusses. The intricacies of who can or will practice (open) science on a social access are almost entirely absent.

2.1.2 *Feminist Open Science(s)*

Who gets to create?

Generally, white men. In the free software world, men outnumber women to a much greater degree than in more traditional development structures.[8] Minorities fare even more poorly.

The dismal representation in FLOSS derives in part from a flawed perception that social stratifications and classifications "don't matter" on the internet. Code is race- and gender-agnostic (except that, for somewhat obvious reasons, it's *not*). Thus, collaborative, decentralized community structures like those in FLOSS usually fail to include any ways of dealing with societal differences. As a result, misogyny and racism run rampant and often unchecked.

Whether representation is *worse* in open science than science at large is something I don't know yet, but it's probably fair to say that open science carries professional risks, and women and minorities in the sciences tend to disproportionately suffer for taking risks.

Who is it designed for?

I don't know yet! But probably I won't like it.

2.1.3 *global definitions*

Free software and open science are built on European and U.S. legal, moral, and social codes. Probably and definitely an issue, especially when "open science" is supposed to level the playing field but other communities, sciences, etc. are actually consulted in how they would like that leveling to be done.

Relevant citations: Christian [2], Dahdouh-Guebas et al. [3], Gorelick [5], Jolliffe [6]

2.2 OPEN ACCESS AND GLOBALIZATION OF KNOWLEDGE

If indigenous sciences are local sciences, specific to the time and place and users involved, then they may not be generalizable to a mythic global community of "scientists".[2] And *if* we create a system where one of those many sciences is targeted as the right one to be spreading, and making access to it the ultimate success or bridge to success, local knowledges are crowded out and erased. Indigenous sciences lose by virtue of what they are – non globalized, local knowledges – with no excuse to *not* use globalized standardized bits of (scientific) knowledge.

2 Destroy the idea of a global community of scientists. If science is local, there is no homogenous community.

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3 DOING OPEN SCIENCE

3.1 WORKFLOW

"Only at Hampshire would your workflow be part of submitted work."

But that's probably not true – or at least shouldn't be. In academic/lab settings, there's an enormous amount of *things* to manage. References, formatting, collaboration, and data and figures come to mind immediately. With science where the whole process *is* the work, it's crucial that all of that information be logically organized, meaningful, and easy to use.

in other places as well, e.g. corporate settings. Might be interesting, and will probably expand on that later, but I only know my own stuff.

In my case, it also has to run on my Linux distribution (Fedora 20 and soon to be 21, if you were wondering), and is ideally libre-licensed software. Fortunely, the first requirement almost always guarantees the second. It's also important (to me) that my software generates freely-formatted files that don't require specialized software for other people to read and manage; I'm not interested in creating file format walls that aren't completely necessary.

open to committee debate on this point

So, preliminary documentation of how my Div is done.

3.1.1 *References*

In a project that might contain a hundred citations, with twice that number reviewed and ultimately discarded, manually tracking and including paper information is an exercise in procrastination.

So I use Zotero, which gives me: a fantastic proxy service that routes all of my journal requests through a friend's MIT Kerberos account, meaning I never see paywalls anymore. It downloads PDFs, retrieves metadata, and renames the file with an [author] - [title] format. . And it's licensed under the GPL, and runs on Linux! Li

though this also means I don't appreciate what information is locked away

Zotero also generates a .bib tex file on command of a given collection. The generated .bib, plain-text file contains, in a structured and organized way, all of the bibliographic information. It's designed to integrate with the incredibly powerful LaTeX system.

Agood and bad: maybe if it wasn't so easy to download things, I would be more selective about what sources make it into my reference library

3.1.2 *LyX & LaTeX*

The most important two things I've realized about my writing process:

1. I care a great deal about how my work looks, and compulsively fuss over tiny style details; I also want it to be legible.
2. I am not a graphic designer, nor am I one interested in becoming someone who designs text, instead of writes it.

So, I need a writing program (a) makes my work look professional where I (b) cannot and do not need to fuss over *typesetting* details.[1]

1 Other handy benefits: automatic numbering and labeling of figures, references, footnotes, sections, and more, that is kept in sync with the rest of the document.

LyX, my editor and a front-end for the LaTeX typesetting language, does exactly that.

> "LyX is a program that provides a modern approach to writing documents with a computer by using a markup language paradigm, an approach that breaks with the obsolete tradition of the "typewriter concept". It is designed for authors who want professional output quickly with a minimum of effort and without becoming specialists in typesetting. The job of typesetting is done mostly by the computer, not the author; with LyX, the author can concentrate on the contents of his writing...[2]

> "So, the basic idea behind LyX is: specify *what* you're doing, not *how* to do it. Instead of "What You See Is What You Get," the LyX model is "What You See Is What You *Mean*" or "WYSIWYM." It's a powerful idea that greatly simplifies the mechanics of writing documents."
>
> -LyX intro

Generally,

3.1.3 *git and version control*

> "[The wikipedia definition] is 'Version control is a system that records changes to a file or set of files over time so that you can recall specific versions later.' We all deal with version control issues. I would guess that anyone reading this has at least one file on their computer with "v2" in the title. Collaborating on a manuscript is a special kind of version control hell, especially if those writing are in disagreement about systems to use (e.g., LaTeX versus Microsoft Word)." (from **?**, "Git/Github, A Primer for Researchers")

Both my lab notebook and my written thesis work are in git repositories. Every time I make changes – add data or edit a protocol or add a new chapter, git tracks it and I have a permanent record of what work I've been doing. This has the added benefit of easily generating a list of accomplishments, which makes me feel better.

In the case of my lab notebook, I've only just started to figure out how to apply the software development model to a biology model, but I think it'll be really powerful and reliably *functional*. That is, its usefulness as an organizing, recording, and sharing tool will only grow as I learn more about it. For my many, many thesis-related files (e.g. every section is a separate file, all my figures are stored external to the document, all my references are in another folder), git has

3.2 IN PRACTICE

As of October, I've had a functioning open lab notebook (in the sense that other people *could* look at it, not that they have...yet) for 3 months.

2 Sadly for LyX, all documents use "he/his" as the default pronoun. Boo! Hopefully I can fix that with an email to the developer list. Information forthcoming.

It relies almost entirely on tools of the development and software world: files written in HTML markdown format, git for version control, Github as a hosting/sharing platform.

The first really drastic difference is how I keep records. Written lab notebooks have a lot to recommend them – easy to write, easy to draw on, able to handle water and reagent drops. What they don't have going is organization and consistency, which software, and specifically the version control tools used to create software, have in abundance

The advantages of tools from the software world weren't and aren't immediately apparent from a biology standpoint. Transitioning from writing all of my notes to keeping them on my computer (and posting them) initially changed nothing about my workflow. It still went: write initial protocol with the date (with no reference to previous work or intent behind it), run protocol, maybe write or include results of intermediary processes if it seemed absolutely necessary or I had a deadline.

One really strong idea is using the right tool for the job. I've long been frustrated by email as a form of information transfer; there's so many better ways to do scheduling, file transfers, and continuous collaboration that could be easily employed.

It's also been really interesting to see the conversations that come up – people are either completely uninterested, or very very interested in sharing ideas and tools to make science more open and more usable. None of the tools that exist so far are really *perfect* - one friend is uploading all of her data to Dropbox. She has a terabyte of storage because she has a friend.

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4

SEROTONIN, TRANSCRIPTION FACTORS, AND DIFFERENTIATION

"every biological organism
is inherently individual"

Serotonin is 5-hydroxytryptamine (5-HT) , a monoamine neurotransmitter binding 14 classes of receptors, all but one of which are G-protein coupled receptors (GPCRs). With a wide array of receptors distributed across the brain, nearly every basic central nervous system (CNS) function, including mood, cognition, sleep, pain, motor function and endocrine secretion, is in some part modulated by serotonin signalling. As such, disruptions in serotonergic transmission is implicated in many psychological diseases (eg schizophrenia etc), as well as some of the mechanisms behind the relevant therapeutic drugs.[7]

Model systems for serotonergic signalling are fundamental to drug design and development for depression and whatever the other stuff is that serotonin does, and in the case of hallucinogenic drugs, present an uncommon means to explore consciousness because of their unique signal transduction specificity.

obviously hallucinogens are what I want to write abut but it's very far away from what I'm doing in lab, so not clear on how they'll get in yet

4.1 MODEL SYSTEMS: E.G. THE INTENTION FOR THE WHOLE LAB BIT

If I want to study second messenger signalling pathways, transfecting cells with the cloned receptor doesn't make sense, because the messenger pathways wouldn't necessarily be consistent with fully differentiated neurons.

So ideally, you'd use primary neuron cultures to study signalling – but those are expensive and hard to maintain.

Thus, development of a holistic serotonin model systems – granted, transgenes are still involved, but they should set cell fate early and thus promote full GPCR signalling.

Be nice.

Also, cell differentiation is cool. So how can we manipulate the P19 stem cells earlier in the process? We can (hopefully) set cell fate early on via expression of important transcription factors, and then allow cells to develop somewhat normally.

4.2 THE 5-HT SYSTEM

1. nomenclature

The 5-HT system is one of the most complex projection networks in the CNS. It has three descending efferents to the spinal cord, while the B5-B9 neurons project via 5 different routes to almost every region of the CNS. This large network means serotonin signalling not only transmits information, but plays a large role in *modulating* the rest of brain function.

1. "most complex projection network in the CNS" – three major descending 5-HT efferents from B1-B4 project to the spinal cord,

Genes	Expression	5–HT defects	KO lethality	5–HT-specific TF
Nkx2.2	VZ	100% except in r1 P0	P0	lost except in r1
Mash1	VZ	Almost 100%	P0	all lost
Gata2	VZ, postmitotic	100%	E10-12.5	all lost
Lmx1b	Postmitotic	100%	P0	all lost
Pet1	Postmitotic	70%	mostly viable	unknown
Gata3	Postmitotic	mostly in caudal	E11.5-13.5	unknown

Table 1: Summary of the phenotype of knockout mice lacking individual transcription factor in 5–HT neurons. (from Chen and Ding)

whereas B5-B9 neurons project through 5 ascendingrouts to almost every region of the CNS"

a) because of these projections, serotonin modulates p. much everything

4.2.1 *embryonic development*

1. distinction b/t future groups unclear
2. among the earliest born CNS neurons
3. rostral 5-HT neuron formation precedes caudal;
   a) ventral motor neurons (vMNs) derived from same domain
4. induction and specification may rely on sonic hedgehog (Shh) which acts through transcription factors
5. **Nkx2.2!** (Nkx2.2!) specifies ventral cell types in response to Shh; may specify 5-HT in hindbrain

4.3 TRANSCRIPTION FACTORS EXPRESSED IN 5-HT PROGENITOR CELLS

4.3.1 *Nkx2.2*

1. responds to Shh signals in spinal cord, hindbrain
2. earliest transcription factor required for 5-HT specifications
3. dispensable in r1 5-HT; Nkx2.2 KO neurons derived from r1 are unaffected
4. in the absence of Phox2b, adopts a default pathway to promote 5-HT fate

4.3.2 *Ascl1/Mash1* *

1. homo or heterodimer basic helix-loop-helix protein structural motif (bHLH); mouse homologue of *Drosophila* pro-neural gene Achaete-scute homolog 1 (Ascl1)
2. key fate determinant for many neuron types;
3. co-expressed with Nkx2.2! during vMN and 5-HT production

   a) in hindbrain, only known proneural bHLH expressed in domain of 5-HT progenitor cells

4. required for 5-HT diff via KO mice
   a) vMNs generated normally but all post-mitotic TFs (Ascl1Pet1,Gata2, Gata3 fail to express
   b) loss of proper Notch signalling that leads to 5-HT neurogenesis
5. possesses specific 5-HT characteristics; cannot be replaced by other pro-neural bHLH factors
   a) not *sufficient* to induce 5-HT diff; needs other cofactors (Nkx2.2 + more?)

4.4 TRANSCRIPTION FACTORS IN VENTRICULAR ZONE & POST-MITOTIC 5-HT NEURONS

4.4.1 *Gata2, Gata3*

1. Members of the GATA family containing zinc-fingers which bind to core (A/T)GATA(A/G)
2. Gata2 precedes -3;
   a) E9.0 in r4 and transiently in r2
   b) E10.5 expanded to all rhombomeres and detected in VZ progenitor cells and in post-mitotic cells
3. Gata2 KO:
   a) severe anemia; death between E10 and E11
   b) defects in neurogenesis generally; early lethality precludes 5-HT specific examination
   c) *in vitro* test; KO tissues lack all 5-HT neurons, even in presence of Gata3
4. Gata2 affects different 5-HT cluster development differently
   a) necessary and sufficient in r1
      i. capable of inducing other 5-HT specific TFs in r1
   b) necessary but not sufficient
5. Gata3 - relevant in caudal development of raphe nuclei
6. conflicting reports of Gata2 + Gata3 interactions
   a) Gata3 operates either independently or downstream of Gata2 and Lmx1b
   b) poorly understand epistatic relationships

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