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A debate never finished - Animal experimentation as a result of an ongoing ethical thought process

CRISPR/Cas9 - facts, prospects and ethical concerns

ETHICS IN SCIENCE AND PUBLICATION II

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Preface

Editorial Note

Dear Reader,

In our second last issue we had a glimpse at the ethical boundaries in science. Dr Corina Gericke shared her opinion on animal testing along with the philosophy of the German association Ärzte gegen Tierversuche e.V. (Doctors Against Animal Experiments Germany). To every voice, howerver, there is a counter voice. Therefore, we logically should shed a light on the arguments that support animal testing. Can there be any such arguments after all? Read more on page 1 where Dr Roman M. Stilling discusses some facts about animal testing.

Ever since DNA was discovered, the science fiction world is crowded with genetically modified or refined creatures. Not only fictious but also real scientist design DNA to improve crop production or drugs. The CRISPR/Cas9 method can be used to artificially alter the genome sequence and is surely the most exciting and promising way to feed and medically support billions of humans. Shady scientists consider the enhancement of the human body itself. Read more about this controversy in Alexander Kronenberg's essay on page 4.

Our readers might notice that we have redefined the section "Questions of the Week" to "Questions of the Month". With this we hope to be able to provide our curious readers regularly with even more thoroughly elaborated curious and inexplicable scientific questions.

With our two issues on ethics in science the editorial board of JUnQ hopes to offer the readers a possibility to form a differentiated opinion on sometimes controversial scientific topics. We cordially invite everyone to share their thoughts and Questions on any scientific topic in a reader's letter which will be published and (hopefully) answered in the following issues. And as always: stay curious and dig through the JUnQ to find the hidden treasures!

—Tatjana Daenzer

Opinions

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A debate never finished
Animal experimentation as a result of an ongoing ethical thought process

Dr. Roman Stilling

Roman Stilling graduated with a B.Sc. in Biosciences from the University of Münster in 2008 and received a Ph.D. degree from the International Max Planck Research School for Neurosciences / University of Göttingen in 2013. Afterwards he joined the APC Microbiome Ireland in Cork, Ireland, as postdoctoral researcher. Since 2016 he is the scientific officer for for the information initiative "Tierversuche verstehen"[1], coordinated by the Alliance of Science Organisations in Germany.

[1] https://www.tierversuche-verstehen.de

Ethical concerns on using animals in biomedical research have been raised since the 19th century. For example, in England the "Cruelty to Animals Act" was passed in 1876 as a result of a debate especially on the use of dogs under inhumane conditions such as invasive physiological experiments or demonstrations without general anaesthesia. Interestingly, it was Charles Darwin who put in all his scientific and political gravitas to push for regulation by the law while at the same time providing highly differentiated argumentation towards using animals for advancing knowledge, especially in the quickly developing field of physiology [1,2]. In an 1881 letter to a Swedish colleague he wrote:

"*[...]I fear that in some parts of Europe little regard is paid to the sufferings of animals, and if this be the case I should be glad to hear of legislation against inhumanity in any such country. On the other hand, I know that physiology cannot possibly progress except by means of experiments on living animals, and I feel the deepest conviction that he who retards the progress of physiology commits a crime against mankind.*"[3]

Animal research as a moral dilemma

In this letter Darwin succinctly summarized the ethical dilemma that is the core of the debate on using animals for research: whether we may cause harm to animals if it is necessary to advance science and medicine.
In fact, the ability to suffer is generally accepted as the single most morally relevant criterion when animals are considered as subjects of moral worth. This reasoning is based on the philosophies of Jeremy Bentham who's thoughts on this matter culminated in the aphorism: "*The question is not, Can they reason? nor, Can they talk? but, Can they suffer?*"[4]

Today, animal welfare legislation is based on this notion in most countries, which has fundamental consequences on how different species of animals are protected by these regulations. For example, in the EU, only the use of animals within the taxonomical subphylum *Vertebrata* (i.e. vertebrates) are covered by the respective EU directive.[5] More recently also the use of *Decapoda* (e.g. crayfish, crabs, lobsters) and *Cephalopoda* (e.g. squids, octopuses) falls within this regulation since it is assumed that these animals have a complex enough nervous system to perceive pain and experience suffering.

Most current legislation in industrialized countries acknowledges that animals (not exclusively, but especially those able to suffer) have intrinsic value and a moral status that is different from other biological forms of life such as plants, fungi or bacteria and inanimate matter. At the same time no country has established legislation that considers the moral status of any animal the same as the moral status of a human being - irrespective of the developmental state or status of health of that human being.

Together this reasoning has led to the appreciation, that legislation cannot reflect a general rule of "one size fits all", but a compromise needs to be implemented, where ethical and scientific judgment for each individual experiment or study is made on a case-by-case basis.

Adherence to the 3R-principle is necessary but not sufficient for ethical justification of laboratory animal use

The moral dilemma of inflicting harm on animals to advance knowledge and medical progress was addressed in more detail in 1959, when William Russell and Rex Burch published "*The principles of humane experimental technique*", in which they formulated the now famous 3R-principle for the first time: Replace, reduce, refine.[6]. This principle acknowledges human benefit from animal experiments but provides a guideline to minimize suffering in animals: Only if there is no alternative method to achieve the scientific goal, all measures to reduce the necessary number of animals in a given study, and the best possible conditions to confine suffering to the necessary minimum have been established, an experiment can be considered as potentially ethically justifiable. Meeting the 3R criteria is, however, a necessary but not sufficient requirement for ethical justification of a particular experiment.
Today the 3R-principle is well accepted worldwide[7] as a formula to minimize animal suffering and has become an integral part of EU animal welfare regulations, which have been translated to national law in all EU member states.

Responsibility towards human life and safety – lessons from history

Another key aspect of research involving the use of animals is human safety, especially in the context of medical research on humans. The atrocities of medical experiments on humans in Nazi Germany has led the international community to implement strong protection of human subjects and patients. In addition, drug scandals like the thalidomide birth defect crisis in the 1950s and 1960s have led to profound changes in drug regulations. The results of this process have been condensed in the "Declaration of Helsinki" adopted by the World Medical Association (WMA) in 1964. Importantly, this declaration states that medical research on human subjects is only justified if all other possible sources haven been utilised for gaining information about efficacy and potential adverse effects of any new experimental therapy, prevention or treatment. This explicitly includes information gained from experiments with animals,[8] which has additionally been addressed in a dedicated statement by the WMA on animal use in biomedical research.[9].

In analogy to the Helsinki Declaration, which has effectively altered the ethical landscape of human clinical research, members of the international research community have adopted the Basel Declaration to acknowledge their responsibility towards research animals by further advancing the implementation of ethical principles whenever animals are being used in research.[10] Further goals of this initiative are to foster trust, transparency and communication on animal research.

Fostering an evidence-based public debate on the ethics of animal research

Transparency and public dialogue is a critical prerequisite for a thoughtful and balanced debate on the ethical implications of using animals in potentially harmful experiments. However, a meaningful public debate about ethical considerations is only worthwhile, if we agree on the facts regarding the usefulness of research on animals for scientific and medical progress.

Yet, the contribution of animal models and toxicology testing to scientific and medical progress as well as subject/patient safety is sometimes doubted by animal rights activists. Certainly, in most biomedical research areas, including those that involve animal experimentation, there is room for improvement, e.g. on aspects of reproducibility or translation of results from bench to bedside. However, there is widespread agreement among researchers and medical professionals, together with a large body of published evidence, on the principal usefulness of animal models in general. As for all science, constant improvement of models and careful consideration of whether any model used is still state of the scientific art at any given point of time is crucial for scientific advancement. Also the responsibility to avoid animal suffering as much as possible dictates that new scientific methods and models free of animal suffering are developed with both vigour and rigour.

A fruitful debate needs to be based on these insights and evidence-based common ground needs to be established when discussing ethical considerations and stimulating new ideas. Finally, we need to acknowledge that we are always in the middle of a continuing thought process, in which we very democratically and carefully need to negotiate the importance of different views, values and arguments.

Read more:

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[2] Feller, D. Dog fight: Darwin as animal advocate in the anti-vivisection controversy of 1875. *Stud. Hist. Philos. Sci. Part C Stud. Hist. Philos. Biol. Biomed. Sci.* **40**, 265-271 (2009).

[3] Darwin, C. R. 1881. Mr. Darwin on Vivisection. The Times. (18 April): 10. (1881). Available at: http://darwin-online.org.uk/content/frameset?pageseq=1&itemID=F1352&viewtype=text. (Accessed: 25th October 2017)

[4] Bentham, J. *An Introduction to the Principles of Morals and Legislation.* (W. Pickering, 1823).

[5] *DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the protection of animals used for scientific purposes.* **2010/63/EU**, (2010).

[6] Russell, W. M. S. & Burch, R. L. *The principles of humane experimental technique.* (Methuen, 1959).

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[9] WMA - The World Medical Association-WMA Statement on Animal Use in Biomedical Research. Available at: https://www.wma.net/policies-post/wma-statement-on-animal-use-in-biomedical-research/. (Accessed: 29th November 2018)

[10] Basel Declaration | Basel Declaration. Available at: https://www.basel-declaration.org/. (Accessed: 30th November 2018)

CRISPR/Cas9—facts, prospects, and ethical concerns

Alexander Kronenberg

Genetic information is encoded in the deoxyribonucleic acid (DNA). In form of a long double-helix molecule, located in living cells, it governs most of the organisms traits. Explicitly, information from genes is used to form functional gene products such as proteins. This process of gene expression is used by all known forms of life on earth to generate the macromolecular machinery for life. Thus, it poses the fundamental level of how the genotype causes the phenotype, i.e. the composite of organisms' observable characteristics. Genomic modification is a powerful tool to amend those characteristics. Reproductional and environmentally caused changes to the DNA is a substrate for evolution. In nature, those changes happen and may cause favourable or unfavourable changes to the phenotype, which allow the cell or organism to improve or reduce the ability to survive and reproduce, respectively.

In the first half of the 20th century, several methods to alter the genetic structure of cells were discovered, which include exposing it to heat, X-rays, UV-light, and chemicals[1-4]. A significant number of crop cultivated today were developed using those methods of traditional mutagenesis, an example of which is Durum wheat, the most prevalent wheat for pasta production. With traditional mutagenesis thousands of mutations are introduced at random within the DNA of the plant. A subsequent screening identifies and separates cells with favourable mutations in their DNA, followed by attempts to remove or reduce possible unfavourable mutations in those by mutagenesis or crossbreeding.

As those methods are usually unspecific and complex, researchers have developed site-determined gene editing techniques, the most successful of which is the so called CRISPR/Cas9 method (clustered regularly interspaced short palindromic repeats). This method borrows from how bacteria defend viral invasion.[6] When the bacterium detects virus DNA invasion, it forms two strands of RNA (single helix molecules), one of which contains a sequence that matches that of the invading virus DNA and is hence called guide RNA. These two RNAs form a complex with a Cas9 protein, which, as a nuclease enzyme, can cleave DNA. When the guide RNA finds the target in the viral genome, the RNA-Cas9 complex will lock to a short sequence known as the PAM, the Cas9 unzippes the viral DNA to which the RNA will match. Cas9 then cleaves the viral DNA, forcing the cell to repair the DNA.[6] As this repair process is error prone, it may lead to mutations that might disable certain genes, changing the phenotype. In 2012 and 2013 it was discovered that the guide RNA can be considerably modified for the system to work site-determined[5], and that by modifying the enzyme it not only works in bacteria and archaea, but also in eukaryotes (plants and animals), respectively.[7]

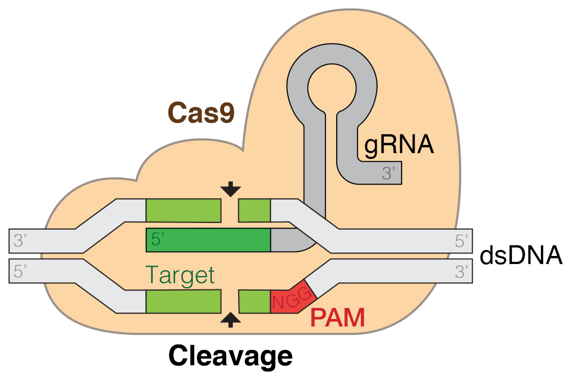

Figure 1: CRISPR/Cas9 working principle.[8]

Research published since demonstrated the method's potential for RNA-programmable genome editing. Modifications can be made so during the repair an artificially designed DNA sequence pairs with the cleaved ends, recombines and replaces the original sequence, introducing new genes to the genome.[11,12] The advantages of this technique over traditional gene editing methods is multifold. It can act very targeted, i.e. site- and therefore gene-specific in any form of known life. It is comparatively inexpensive, simple enough to be conducted in basic labs, effective, and fast regarding preparation and realisation. The production of multiplex genetically modified mice, for instance, was reduced from up to two years to few weeks,[9] as CRISPR/Cas9 has the unique advantage over earlier genome editing methods, that multiplexable targeting is easily achieved by co-expressing Cas9 with multiple single-guide RNAs simultaneously. Consequently, within few years after its discovery, it evolved to be the routine procedure for genome modification of virtually all model plants and animals.

The availability of such a method evokes medical and botanical development interests. A plethora of possible medical applications are discussed and researched, among which is healing cancer or treating genetic disorders. For cancer research it is imaginable to induce a multitude of deliberate mutations to artificially form cells similar to cancerous cell, study the caused modification to the cells, and thus learn to inhibit their reproduction or the original mutation. In the clinical research focus now are blood diseases or those related to haematopoietic cells, such as leukaemia, HBV, HIV, or haemophilia.[13,14] This is because for the treatment of those diseases, the cells (blood cells or bone marrow) can be extracted from the body in a known way, their genome can be edited in vitro by the CRISPR/Cas9 method, and finally the cells can be reintroduced to the body. The advantage of the extraction is that no additional vector (agent to help finding the right cells in vivo) is required, and the genomic modification can be controlled ex vivo. While the editing efficiency with CRISPR-Cas9 can be extremely high, the resulting cell population will be inherently heterogeneous, both in the percentage of cells that were edited and in the specific genotype of the edited cells. Potentially problematic for in vivo application is the bacterial origin of the endonuclease Cas9. A large portion of humans show humoral and cell-mediated immune responses to the Cas9 protein complex,[10] most likely because of prior infection with related bacteria.

Although clinical applications of CRISPR/Cas9 grab a lot of media attention, agricultural applications draw even more commercial interest. Prospects here are the faster, cheaper and more targeted development of crops than by traditional methods of mutagenesis, which are extremely more aggressive in comparison. The main aim is unchanged though: improve plants regarding yield, resistance to diseases or vermin, and resilience to aridity, heat, cold, humidity, or acidity.[15,16] CRISPR/Cas9 is therefore considered an important method to ameliorate agricultural food production to feed the earth's ever-growing human population.

Regulations of thusly modified products vary largely between countries. While Canada considers such plants equal to not genetically modified if no transgene was inserted, the USA assesses CRISPR plants on a case by case basis, gauging whether the modification would have been possible by natural mutation. This way they chose to not regulate mushrooms that do not turn brown and maize with an altered starch contend. Last year the European court of justice ruled all CRISPR/Cas9 modified plants as genetically modified organisms, reasoning that the risks of such a novel method are unknown, compared to traditional mutagenesis as an established method of plant breeding.

Instigated by genome editing in human-embryonic cells in 2015[18] a group of scientists called for a moratorium to discuss the possible risks and impact of the wide usage of the CRISPR/Cas9 technology, especially when it comes to mutations in humans.[19] On the 2015 International Summit on Human Gene Editing leading international scientists considered the scientific and societal implications of genome editing. The discussed issues span clinical, agricultural and environmental applications, with most attention focused on human-germline editing, owing to the potential for this application to eradicate genetic diseases and, ultimately, to alter the course of evolution. Some scientists advise to ban CRISPR/Cas9 based human genomic editing research for the foreseeable future, whereas others favour a rapid progress in developing it.[20] A line of argument of supporters of the latter viewpoint is, that the majority of ethical concerns are effectively based on methodical uncertainties of the CRISPR/Cas9 method at its current status, which can be overcome only with extensive research. Those methodical uncertainties include possible cleavage at undesired sites of the DNA, or insertion of wrong sequences at the cleavage site, resulting in the disabling of the wrong genes or even the creation of new genetic diseases.

Whilst a total ban is considered impractical because of the widespread accessibility and ease of use of this technology,[21] the summit statement says, that "It would be irresponsible to proceed with any clinical use of germline editing unless and until (i) the relevant safety and efficacy issues have been resolved ... and (ii) there is broad societal consensus about the appropriateness of the proposed application." The moral concerns about embryonic or germline treatment base on the fact that CRISPR/Cas9 not only would allow the elimination of genetic diseases, but also enable genetic human enhancement, from simple tweaks like eye colour or non-balding to severe modifications relating bone density, muscular strength or sensory and mental capabilities.

Although most scientist echo the summit statement, in 2018 a biochemist claimed to have created the first genetically edited human babies, two twin sisters. After in vitro fertilization, he targeted a gene that codes for a protein that one HIV variant uses to enter cells, enforcing a kind of HIV immunity, which is a very rare trait among humans.[22] His conduct was harshly criticised in the scientific community, widely condemned, and-after enormous public pressure-redoing forbidden by the responsible regulatory offices.

Ultimately the CRIPSR/Cas9 technology is a paramount example of real world societal implications of basic research and demonstrates researchers' responsibilities. This also raises the question whether basic ethical schooling should be part of every researcher's education.

Read more:

[1] K. M. Gleason (2017) "Hermann Joseph Muller's Study of X-rays as a Mutagen"

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[20] J. Doudna, Nature 528, p. S6.
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[22] Regalado, A. (2018). "Chinese scientists are creating CRISPR babies". MIT Technology Review.

Views on Life, the Universe, and Everything

Questions of the Month

The Journal of Unsolved Questions presents a “Question of the Month” on its homepage every month. Set up and formulated by the members of the editorial board, or guest writers, the main purpose of the “Question of the Month” consists in intriguing the reader by presenting topics of ongoing research. “Questions of the Month” published so far cover a wide variety of scientific fields, but share the feature to be of certain interest to several disciplines.
In the following, we present selected “Questions of the Month” from the last six months.

Why do alkali metals actually explode in water?

Kai Litzius

It is one of the most common educational experiments in school and straight from the books: The reaction of an alkali metal with water. During this reaction significant amounts of hydrogen gas are produced, which can ignite and thus explode due to the strongly exothermic reaction – at least that is the explanation one finds pretty much everywhere. However, there is something odd about this reasoning. On the one hand, a complete immersion of the metal within water should then prevent the explosion from happening as no oxygen is present to ignite the hydrogen gas. On the other hand, it is surprising that the solid-liquid interface of this heterogeneous reaction creates enough physical contact to drive the reaction. Additionally, the produced gas tends to separate the educts and therefore stop the reaction. Overall, there are quite a few unclear details in this proposed reaction mechanism.

A study of the Czech Academy of Sciences in Prague and the Technical University of Braunschweig, however, showed that even in presumably clear textbook reactions a lot of surprises may be found sometimes.[1,2] The scientists used drops of sodium-potassium alloy that is liquid at room temperature and filmed the reaction with high speed cameras. They could show that the explosive reaction also happens under water when the metal is completely immersed, thus ruling out the ignition of the hydrogen gas as the main driving mechanism for the explosion. Supported by molecular dynamics simulations, they instead showed what mechanism actually drives the reaction: A Coulomb explosion! During the reaction of a clean metal surface with the adjacent water molecules, electrons move quickly from the metal atoms into the water. This also explains why a solid piece of an alkali metal does not always explode in water: it needs a clean interface without significant oxidation. After the electrons left the metal surface and moved into the water, a strongly charged surface is left. On this surface, the ionized atoms strongly repel each other, and thus open up a path to more inner atoms that have not taken part in the reaction yet. On a time scale of about 0.1 ms, metal dendrites shoot into the water (see figure) and suddenly increase the surface area of the metal.[1-3] This happens extremely fast with giant charge currents flowing in the interface region. The surface tension is pretty much nullified in this case[2,3] and the expanding surface provides more reactive area. As a result, large amounts of hydrogen gas are suddenly produced. Together, these effects drive the explosion, while the ignition of the gas is not directly necessary for the explosion to occur. Instead, the hydrogen gas can also burn off later.[2]

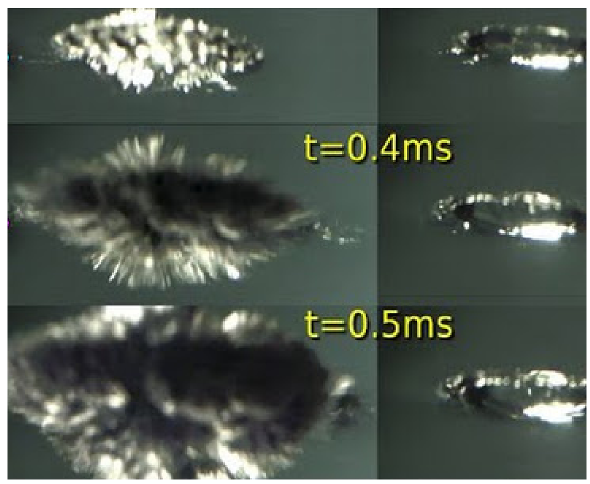

Figure 1: As soon as a drop of NaK-alloy gets in contact with water (top left), fine metal fingers are protruding into the water (middle). These are driven by the Coulomb explosion that massively increases the surface area and therefore the reactive interface. As a result, a fast production of hydrogen becomes possible, which further drives the explosion (bottom left). The right column depicts the impact of a water droplet for reference. Figure is taken from the YouTube cover image of ref.[3]

Further results of the study could lead to approaches to avoid metal-water explosions and thus gain application relevance in industry. What is however most unusual about this study is that parts of it got funded by the YouTube science channel of the lead author of the paper, which he explicitly acknowledges. In this exciting case, science and media are really in a close relationship.

Read more:

[1] P. E. Mason et al., Nature Chemistry 7, 250–254 (2015)

[2] https://youtu.be/LmlAYnFF_s8 (last access 12/01/2018)

[3] https://youtu.be/xMfQSV4ygHE (last access 12/01/2018)

How many colours are needed for a map? And for a plane?

Alexander Kronenberg

When Francis Guthrie took on the task to colour a map of England in 1852 he needed four colours to ensure that no neighbouring shires had the same colour. Is this the case for any map imaginable, he wondered.
As it turns out, five colours do suffice, as mathematically proven in 1890 in the five-colour theorem.[1] That indeed four colours are enough to colour a map if every country is a connected region took until 1967 to prove and required computer assistance.[2] It abstracted the idea to geometric graph theory where regions are represented by vertices connected by an edge if they share a border (see fig. 1).

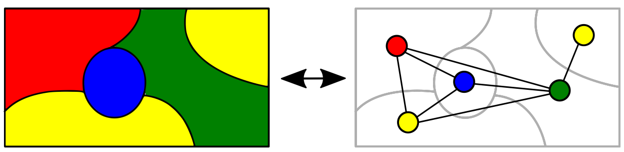

Figure 1: Illustration of the abstraction of the map colouring problem to graph theory.

The four-colour theorem was then proven by demonstrating the absence of a map with the smallest number of regions requiring at least five colours. In its long history the theorem attracted numerous false proofs and disproofs. The simplest versions of counterexamples focus on painting extensive regions that bordering many others, thereby forcing the other regions to be painted with only three colours. The focus on the large region might cause people's inability to see that colouring the remaining regions with three colours is actually possible.
Even before the four-colour theorem was proven, the abstraction to graph theory evoked the question as to how many colours would be needed to colour a plane so that no two points on that plane with distance 1 do have the same colour. This is also known as the Hadwiger–Nelson problem. Note that we are not colouring continuous areas in this case, but instead each individual point of the plane, rendering it extremely more complex. In the 1950s it was known that this sought number, the chromatic number of the plane, had to be between four and seven.
The upper border is known from the existing tessellation of a plane by regular hexagons that can be seven-coloured[4] (fig. 2). The maximal distance within one hexagon, the diameter, needs to be smaller than one to comply with the requirement. Additionally one needs to ensure that the distance to the next hexagon of the same colour is larger than one. These constraints imply that the hexagon edge length a has to be between 0.5 and $\sqrt{(7)}/2$ for an allowed colouring of the plane, where no two points with distance one have the same colour.

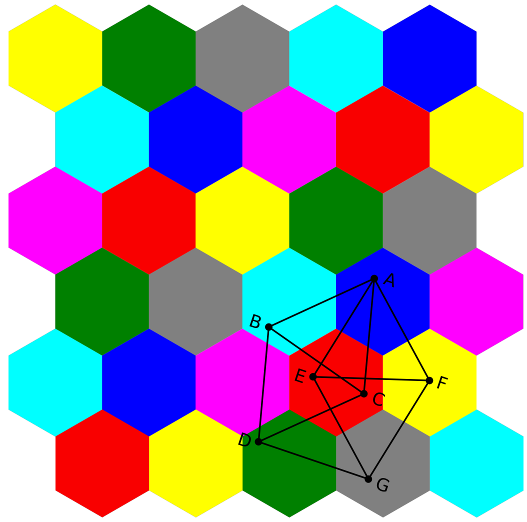

Figure 2: Colouring if a plane in a seven colour tessellation pattern of regular hexagons.

As to the lower border for the chromatic number of the plane, it is obvious that two colours will not suffice to colour even the simple unit-distance path of an equilateral triangle (see fig. 3 a). To demonstrate that three colours do not suffice either and therefore at least four colours a needed, we take a look at the Moser spindle shown in fig. 3 b. The seven vertices (all eleven edges / connections have unit-distance) cannot be coloured with three colours, say green, blue, and yellow. Assigning green to vertex A, its neighbours B and C need to be blue and yellow, respectively, or vice versa, enforcing D to be green again. A's other neighbouring vertices E and F analogously are assigned blue and yellow, or vice versa, enforcing in turn G to be green. This conflicts with G's neighbour D to be green, too, thus demonstrating that arbitrary unit-distance graphs require at least four colours.

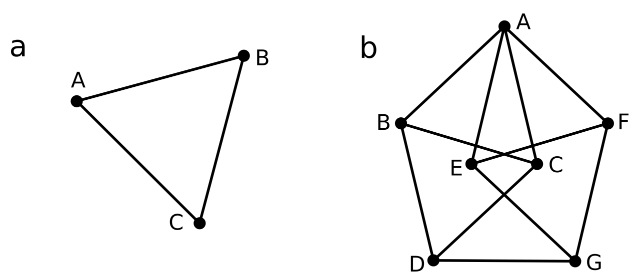

Figure 3: a) An equilateral triangle as a simple example for a unit-distance graph. b) The Moser spindle is a four-colourable unit distance graph.[3]

After many years of intractability only this year there was some significant progress in closing in on the Hadwiger–Nelson problem. It was demonstrated that "the chromatic number of the plane is at least 5"[5], by finding two non-four-colourable unit-distance graphs (with 20425 and 1581 vertices). The smallest unit-distance graph with chromatic number five found this year has 553 vertices[6] and is shown in fig. 4. Whether the chromatic number of the plane is five, six, or seven still remains to be shown.

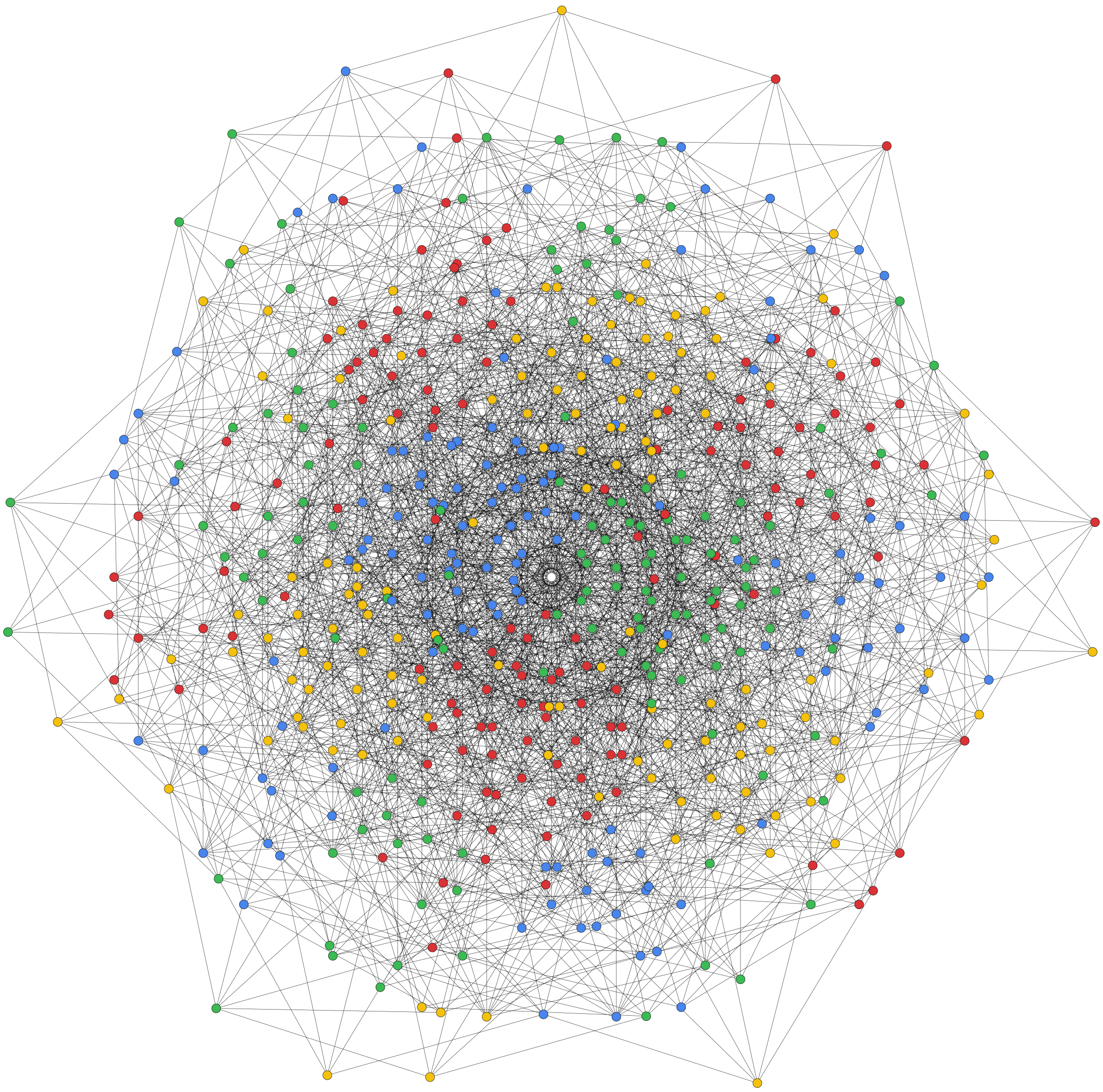

Figure 4: Five-colourable unit distance graph with 533 vertices. The fifth colour (white) is only used in the centre.[6]

Read more:

[1] Heawood, (1890), “Map-Colour Theorems”, Quarterly Journal of Mathematics 24, pp. 332–338

[2] Appel, Haken, (1989), “Every Planar Map is Four-Colorable”, Contemporary Mathematics 98, With the collaboration of J. Koch., doi:10.1090/conm/098

[3] Soifer, (2009) “The Mathematical Coloring Book”, Springer

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[6] Heule, (2018), “Computing Small Unit-Distance Graphs with Chromatic Number 5”, arXiv:1805.12181

How do you turn your spaghetti?

Tatjana Daenzer, Mariia Filianina, Alexander Kronenberg, Kai Litzius, Adrien Thurotte

Certainly, most of us enjoy an occasional nice bowl of spaghetti. Some of us use a spoon along with the fork, some don't. Doesn't matter, as long as you enjoy and don't make a mess. But have you ever wondered whether there is a preferred direction to turn the fork? And is it related to where you live? We did! In our last issue (Vol 2, 2018), we launched a survey asking our readers exactly this question (Figure 1).

In which direction do you turn your fork when eating spaghetti?

- ☐ I am right-handed and turn clockwise
- ☐ I am right-handed and turn counterclockwise
- ☐ I am left-handed and turn clockwise
- ☐ I am left-handed and turn counterclockwise
- ☐ I have no preferred direction
- ☐ I shovel

Where do you live?

- ○ I live on the northern hemisphere
- ○ I live on the southern hemisphere

Figure 1: The Spaghetti Turn survey as it appeared on the webpage http://junq.info/?p=3550.

Our survey was advertised in social media (Facebook, LinkedIn, Twitter, ResearchGate) and via QR codes on flyers. The survey reached a total number of n=158 readers, 132 of them found their way directly to our website. The results are shown in the table below and Figure 2.

The option „no preferred direction" remained unanswered. One single participant chose "I am right-handed and turn clockwise" and "I am right-handed and turn counter clockwise", depicted as "other". Assuming that this is no miss-click one out of a total number of 160 participants has no preferred direction when using the fork with their right hand. This underlines that most people on earth indeed have a favourite direction to screw the fork.

Although there is no clear definition to determine handedness, some publications claim that 70–95% of human population worldwide are right-handed, 5–30% are left-handed and a small minority is ambidextrous.[1] This is consistent with our findings: the survey was answered by 133 right-handed people, which is 86.9% of all 154 participants who revealed their handedness. 20 participants are left-handed (13.1% of all 154 participants who revealed their handedness). One participant (<1%) is ambidextrous and turns the fork counter clockwise with both hands.

	Northern Hemisphere		Southern Hemisphere		worldwide	
	n	%	n	%	n	%
right-handed clockwise	117	75.5	3	60	120	75.0
right-handed counter clockwise	12	7.7	1	20	13	8.1
left-handed clockwise	10	6.5	0	0	10	6.3
left-handed counter clockwie	10	6.5	0	0	10	6.3
left-handed clockwise	0	0	0	0	0	0
left-handed counter clockwie	1	0.6	0	0	1	0.6
showel	4	2.6	1	20	5	3.1
other	1	0.6	0	0	1	0.6
sum	155	96.9	5	3.1	160	100

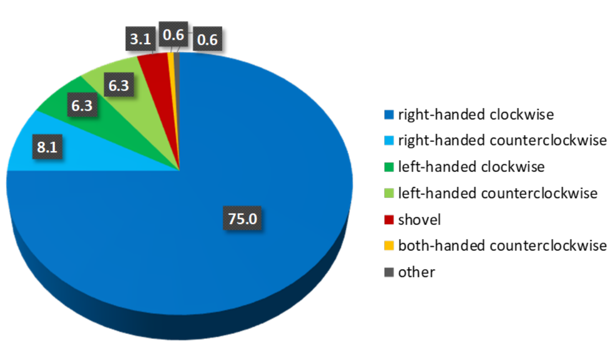

Figure 2: Worldwide percentage of the preferred direction to turn the fork when eating spaghetti related to the handedness (values in %)

75.0% of all participants are right handed and turn the fork in clockwise direction. Only 8.1% turn it counter clockwise. Surprisingly, there seems to be no preference about the turning direction among left-handed people. Their numbers equal (each ten or 6.3%), while 90.2% of all right-handed people turn clockwise. Fortunately (or shockingly?), 3.1% of spaghetti eaters worldwide shovel.
Unfortunately, we did not reach a significant number of readers from the southern hemisphere. Four participants out of five are right-handed, one shovels. 60% of the right-handed southerners turn the fork clockwise, 20% turn it counter clockwise. The results from the northern hemisphere do not drastically differ from the whole world. Considered that only five participants (3.1% of all) do not represent the whole 10% of the human population living on

the southern hemisphere,[2] the preference of turning counter clockwise shows a similar tendency for both hemispheres. There is therefore supposedly no relation to where you live on this planet.

But why is the clockwise direction so obviously favoured? Time and therefore clocks have a powerful influence in our daily lives. Also, in a lot of cultures texts are written from left to right (as the clockhand moves). Moving and looking to the right is very often linked to the future and openness. An experiment from Sascha Topolinski and Peggy Sparenberg from 2012 suggests, that the preferred direction to turn objects could be determined by one's conservative or open personality.[3] Or is it just for handling reasons only and it is a little easier to apply force on the edge of the fork while turning it clockwise? With a simple survey like our's it is impossible to determine whether the habit to turn the fork left or right is a matter of education, subconsciousness or technique.

Throughout the active survey it was possible to answer the poll via the Facebook "Surveys for Pages" and our webpage. Hence, we cannot entirely assure the integrity of the results. Also, we hope our readers understand humour but also answer the survey genuinely. We simply trust in the scientific spirit of our readers. We also did not consider that for cultural habits in certain cultures spaghetti dishes might not be available or forks might not be part of the traditional cutlery. Although it is very often a cause for heavy crossfires during meals, the use of a spoon along with the fork is discounted in the evaluation of the results too. With this survey we just aim to give a picture about the general turning behaviour of spaghetti eaters. To the best of our knowledge there has not been a similar survey until now.

We are now smarter than before but still missing the details of the big picture. Let's see what the new year brings...

Read more:

[1] https://www.scientificamerican.com/article/why-are-more-people-right/ (last access 31.12.2018)
[2] https://bigthink.com/strange-maps/563-pop-by-lat-and-pop-by-long?page=all (last access 31.12.2018)
[3] Sascha Topolinski, Peggy Sparenberg, Social Psychological and Personality Science, 2012, 3, 308–314

Which way is up?

Mariia Filianina

Imagine you are on an airplane, ten thousand meters up in the sky. Now, if you close your eyes you know exactly which way the airplane has started moving, whether it has begun to manoeuvre to the right or to descend. This ability we owe to our inner ear as a part the humans' vestibular system.

The vestibular system is designed to send information about the position of the head to the brain's movement control centre, that is the cerebellum. It is made up of three semi-circular canals and two pockets called the otolith organs (Fig. 1), which together provide constant feedback to the cerebellum about head movement. Each of the semi-circular canals is orthogonal to the two others so that they detect the variety of movements in three independent directions: rotation around the neck (horizontal canal), nodding (superior canal) and tilting to the sides (posterior canal). Movement of fluid inside these canals due to the head movement stimulates tiny hairs that send signals via the vestibular nerve to the cerebellum. The two otolith organs (called the saccule and utricle) signal to the brain about linear movements (backwards/forwards or upwards/downwards) and also about where the head is in relation to gravity. These organs contain small crystals that are displaced during linear movements and stimulate tiny hairs communicating via the vestibular, or balance nerve to the cerebellum.

So why is that, even equipped with such a tool, sometimes we get a feeling sitting on an airplane that it is falling down when in fact it is not? Why is that some people, particularly underwater divers, may lose direction and no longer know which way is up?[1] Surely, typical divers should still have the inner ear, unless a shark has bitten their heads off. Is it all caused by stress? Actually, there is much more to it!

Humans have evolved to maintain spatial orientation on the ground, whereas the three-dimensional environment of flight or underwater is unfamiliar to the human body, creating sensory conflicts and illusions that make spatial orientation difficult. Normally, changes in linear and angular accelerations and gravity, detected by the vestibular system, and the relative position of parts of our own bodies, provided by muscles and joints to the proprioceptive system, are compared in the brain with visual information. In unusual conditions, these sensory stimuli vary in magnitude, direction, and frequency. Any differences or discrepancies between visual, vestibular, and proprioceptive sensory inputs result in a sensory mismatch that can produce illusions. Often the result of these various visual and nonvisual illusions is spatial disorientation.

For example, fighter pilots who turn and climb at the same time (they call it "bank and yank"), feel a strong sensation of heaviness. That feeling, caused by their acceleration, surpasses the pull of gravity. Now, if you asked them while blindfolded to tell which way was down using only their vestibular organ, they would point to the cues provided by the turn, not to the cues provided by the earth's gravity.[2]

Furthermore, the vestibular system detects only changes in acceleration, thus a prolonged rotation of 15-20 seconds[3] results in a cessation of semi-circular output. As a result, the brain adjusts and does not feel the acceleration anymore which can even result in the perception of motion in the opposite direction. In other words, it is possible to gradually climb or descend without a noticeable change in pressure against the seat. Moreover, in some airplanes, it is even possible to execute a loop without exerting negative G-forces so that, without visual reference, the pilot could be upside down without being aware of it.

Another interesting example is the phenomenon of loopy walking. When lost in a desert or a thick forest terrain without landmarks people tend to walk in circles. Recent studies performed by researchers of Max Planck Institute for Biological Cybernetics, Germany, revealed that blindfolded people show the same tendency. Lacking external reference points, they curve around in loops as tight as 20 meters in diameter while believing they are walking in straight lines.[4]

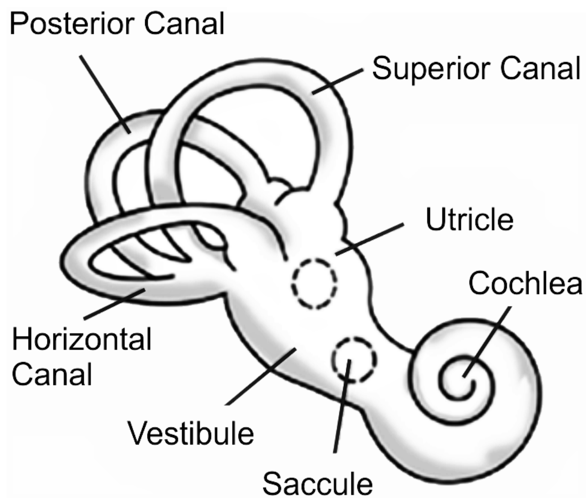

Figure 1: Schematic structure of a humans' inner ear.[5]

Seemingly the vestibular system is quite easy to trick by eliminating other sensory inputs. However, even when visual information is accessible, e.g. underwater, spatial disorientation can still occur [any scuba diving forum – for the reference]. The obvious fact that water changes visual and proprioceptive perception is crucial here: humans move slower, see differently and let's not forget the Archimedes' principle. It happened a lot, that a confused diver thought that the surface was down, especially when the bottom seemed brighter because of reflections. This can be a dangerous mirage in such an unusual gravity. On top of it, water can affect the vestibular system directly through the outer ear. When the cold water penetrates and reaches the vestibular system, it can cause thermal effects on the walls of the semi-circular canals, leading to slight movements of the fluid inside, which are enough to be detected by the brain. Just like in the situations described before this causes the symptoms of spatial disorientation and dizziness.

The vestibular system is indeed frightfully complicated. We can trick it for fun riding roller coasters in an adventure park, but when incorrect interpretation of the signals coming from the vestibular system occurs at the wrong moment this can lead to serious consequences. Luckily, nowadays the airplanes and even divers are equipped with precise instruments used to complement the awareness of the situation and thus avert dangerous situations.

P.S. If you are interested, try riding an elevator while seated on a bike.

Read more:

[1] The Editors of Encyclopaedia Britannica, (2012). Spatial disorientation, Encyclopædia Britannica, inc.

[2] L. King, (2017). The science of psychology: An appreciative view. (4th. ed.) McGraw-Hill, New York

[3] Previc, F. H., Ercoline, W. R. (2004). Spatial disorientation in aviation. Reston, VA: American Institute of Astronautics and Aeronautics

[4] J. L. Souman, I. Frissen, M. N. Sreenivasa and M. O. Ernst,Walking straight into circles, Current Biology 19, 1538 (2009)

[5] https://commons.wikimedia.org/wiki/File:Balance_Disorder_Illustration_A.png

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