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ScienceMind

SUMMER 2022

KING'S College LONDON

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Alexi Mery
Amina Igenbek
Andres Jaramillo Moyano
Anmoyul Mohon
Annika Robiolio
Cansu Ozdemir
Devina Patel
Dima Aimuhtaseb
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Kalyan Ghadiyaram
Leon Zhang
Olivera Mitevska
Prachi Puranik
Rosa Tsucala
Vidur Tandon
Zeta Ioannou

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Amina Igenbek
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Emma von Seth
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Jenna Keung
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Sichun Yao
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Yusra-Aina Choudhury

THIS ISSUE

Dear Reader,

This is my first issue as Editor in Chief of ScienceMind and I can't wait for you to read the articles that the writers have put together. Special thanks to everyone on the team that worked hard over the summer to bring this magazine to life. This issue has an article for all scientific interests, and the categories this month include: immunology, oncology, medicine, cardiology, neuroscience, pharmacology, technology, sports science, synthetic biology, and business/law. We are featuring an interview conducted by Alexi Mery with leading cognitive neuroscientists Andreas Nieder and Nicky Clayton, specialising in animal cognition. If you want to know whats it like to be a crow, learn more about a potential cure for cancer, whether robots can feel pain, and a lot more, this issue is for you.

If this is your first time reading our magazine...
Science Mind is the award-nominated, student-led science magazine of King's College London. We report the latest findings in STEM to students and the wider community. We showcase and develop the written and oral communication skills of students interested in STEM by concisely explaining complex scientific concepts in the form of lay articles and by conducting interviews. Authors can also broaden their knowledge by writing articles for different sectors between issues.

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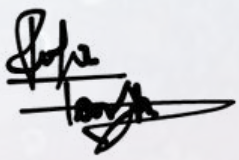

The Editor-in-Chief
Rosa Tsucala

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SHALLOW DIVE

mRNA COVID-19 vaccine impact on the immune system

WRITTEN BY HAY YEE YUAN, HARMONY
EDITED BY HUDA HAMMAD RATTU
DESIGNED BY JENNA KEUNG

COVID-19 is a threatening infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-COV-2). Research on vaccine development has been done, where mRNA vaccines have shown propensity to be successful against this pandemic. With this in mind, the study of COVID-19 vaccines on a molecular level is crucial to expand the research in vaccination development for Covid or other medical conditions in the coming years. This article will first describe the SARS-COV-2 structure & the mechanism of the mRNA vaccine. Then, the effects of COVID-19 mRNA vaccines on the body's immune system at a molecular level will be discussed.

The severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) is part of the coronavirus family that leads to COVID-19. S, E, M, and N proteins are present on the lipid membrane of the virus, their functions are generally stated in Fig.1.

.

mRNA vaccines train body cells to develop SAR-COV-2's spike (S) protein to initiate an immune response. Once the immune response is triggered, B-lymphocytes and T-lymphocytes are produced for antibody production and for killing infected cells respectively.

Fig. 2 shows a general overview of the mechanism of the mRNA vaccine after injection. The recombinant mRNA first enters the cytoplasmic compartment via endocytosis. The mRNA will then be translated into corresponding proteins. The derived protein acts as an endogenous antigen and will be broken down to generate antigenic peptides. The antigenic peptides are then presented to the CD8+ cytotoxic T cell (cytotoxic T cells) using the major histocompatibility complex (MHC) class I molecular pathway, followed by the cell-mediated immune response.

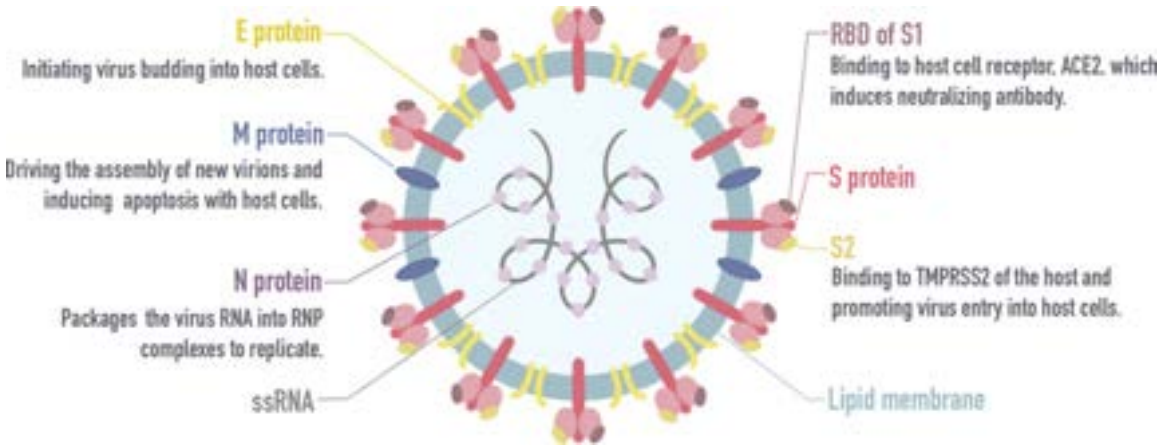

Figure 1. Structure of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and explanation of components . Adapted from Wang et al.,2022.

On the other hand, some exogenous antigenic peptides will be presented to the CD4+ T cells (helper T cells) via the MHC class II molecular pathway to elicit the humoral response. As a result, antibodies will be produced by the B cells that are activated by CD4+ T cells.

This mechanism is used for COVID vaccinations where mRNA codes for the spike (S) protein of SARS-COV-2. Although mRNA vaccine is only available until recently, it has been studied for a long period of time. There are a few known advantages that shape the current success in COVID-19 mRNA vaccines. First of all, possible COVID-19 symptoms that may arise in traditional vaccination could be eluded since mRNA vaccines do not use live viruses. Furthermore, it is shown that the mRNA will not interact with the DNA of the cell. This means body cells' DNA will not be affected or changed to avoid other harmful effects.

The positive impacts of mRNA vaccines are studied by scientists at a molecular level for a better understanding for future vaccination developments. This article will discuss one of the pilot studies* about COVID-19 mRNA vaccines and explain the effects of mRNA vaccines on the immune system at a molecular level.

A recent pilot study researched the types of antibodies observed and analysed in people who received their vaccination. In this research, two stratified approaches - the presence/lacking of a positive COVID-19 history and the presence/absence of the side effects after vaccination are used. Blood samples are taken from the subject and examined using multiple methods.

*A pilot study is a study with a small sample size

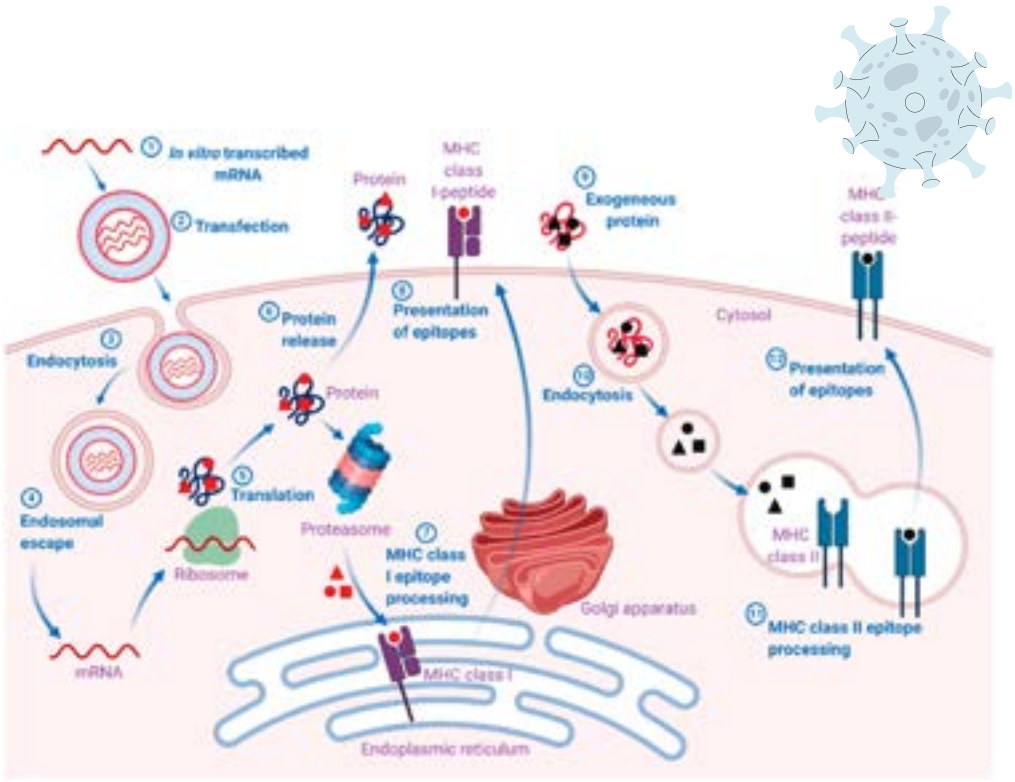

Figure 2. Mechanism of action of mRNA vaccines. Adapted from Wadhwa et al., 2020.

Immunoglobulin G (IgG), Immunoglobulin A (IgA), S2 antibody and N antibodies are identified in blood samples of individuals. Interferon Gamma (IFN-γ) is also identified from subject's blood samples using the Interferon Gamma Release Assay (IGRA). Using the first stratification approach (covid history), it is suggested that IgG class antibodies will not differ from individuals who have or lack a positive covid history. However, subjects with positive covid history have better memory responses for IgA class antibodies to the peptides of SARS-COV-2. Furthermore, S2 antibody levels are higher in individuals with a positive covid history, corroborating the effect of coronavirus exposure to the S2 antibody response to the mRNA vaccine. N antibodies are observed to have the tendency to be more present in individuals with a positive COVID-19 history. Last but not least, the study indicates a high level of IFN-γ in the IGRA assay. This indicates the importance of T-cell activation for a more effective mRNA vaccine.

Although further investigations are still required, this study might offer some directions for future mRNA vaccination research to improve people's quality of life.

References

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TREADING WATER

The Association between Allergic Rhinitis and Alzheimer's Disease

WRITTEN BY WAI IN NG, JANINE
EDITED BY ZETA IOANNOU
DESIGNED BY JENNA KEUNG

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders that contributes to the impairment of cognition and behaviour. It is characterised by key symptoms including memory loss and dementia. Some of the distinctive pathophysiological features of AD are extracellular amyloid plaques and intracellular neurofibrillary tangles with tau proteins. These aggregated and misfolded proteins accumulate in brain lesions (Muchowski, 2002).

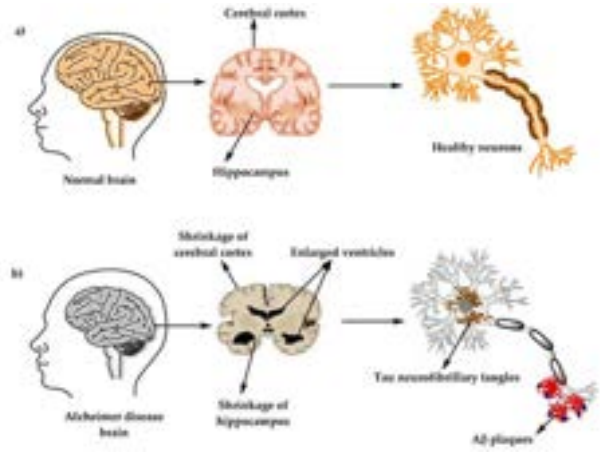


Figure 1. 1. The physiology of neurons in (a) a healthy brain and (b) a brain of a patient with Alzheimer's disease (AD). Adapted from Breijyeh and Karaman, 2020.

The prevalence of AD is observed in Allergic Rhinitis (AR) patients. AR is another disease prevalent among the global population that affects the respiratory system. The symptoms of this IgE-mediated inflammatory disease are nasal congestion, sneezing and anterior nasal leakage. The problem of an ageing population and increasing exposure to allergies, have resulted in a growing number of elderly patients affected by atopic diseases and possibly with concomitant AD. The time has come to analyse if AR increases AD risk besides solely exploring the epidemiology, diagnostics and treatment of AR.

AD is perceived as a multifactorial disorder, with the cholinergic hypothesis being proposed as one of its main causes. Acetylcholine (ACh) is a neurotransmitter involved in cognitive functions ranging from sensory formation to memory. One of the crucial roles of ACh is to enhance memory formation and consolidation by modulating hippocampal and cortical synaptic plasticity (Breijyeh & Karaman, 2020). In other words, the usage of anticholinergics, which inhibit the ACh binding onto muscarinic ACh receptors, may lead to cognitive decline.

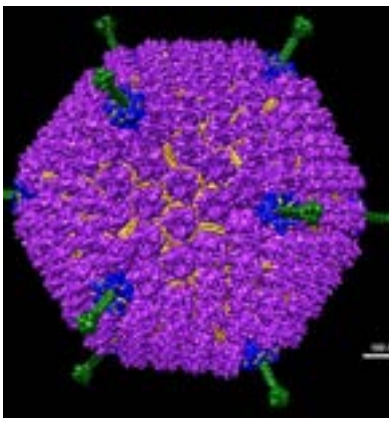
First-generation antihistamines for AR patients also have anticholinergic effects. Due to their ease of crossing the blood-brain barrier, the drugs have the potency of sedation, which may give rise to cognitive impairments in memory and learning. Moreover, histamine H1 receptors and muscarinic ACh receptors demonstrate a homology of at least 30%. This indicates that the amino acid sequences of both types of receptors are of significant similarity. With their low selectivity toward H1 receptors, first-generation antihistamines inhibit cholinergic transmission (Yanai, 2012). Yet, second-generation antihistamines such as cetirizine and desloratadine do not demonstrate anticholinergic action owing to their selective H1 blockade (Yilmaz & Corey, 2006). This could serve as a possible method to reduce the occurrence of AD in AR patients.

Elderly people tend to be more sensitive to anticholinergic activities in the central nervous system. This sensitivity could be attributed to age-associated changes such as an increased blood-brain barrier permeability and decreased ACh-mediated transmission in the brain, which may bring about the progression of AD itself (Malaz Boustani, 2009).

It is suggested that air pollution, which exacerbates AR, may eventually cause AD. A recent study in Taiwan shows that an increase in the PM2.5 exposure level could increase the probability of AD in AR patients (Li et al., 2019). AR can be developed from inflammation in the olfactory mucosa under exposure to environmental allergens. They trigger oxidative stress in the olfactory epithelium, leading to protein misfolding and neuronal apoptosis. These allergens also play a role in microglia recruitment that may enhance the release of proinflammatory cytokines such as TNF-α, IL-β or IFN-γ, one of the most important features of AD pathology (Son et al., 2021). Nevertheless, further research on the olfactory immune system is required to advance our understanding of the pathogenesis and progression of AD due to limited clinical data available.

Most of the interpretations in the literature are on the basis of similar mechanisms. Few studies on the relationship between AR and AD are conducted at the time of writing, so the relationship remains unclear; yet, the prevalence of AD among AR patients should not be overlooked.

References



CAR T-CELLS AND ONCOLYTIC VIRUSES VERSUS CANCER: A LIFE SAVING COMBINATION

WRITTEN BY VIDUR TANDON
EDITED BY ZETA IOANNOU
DESIGNED BY ALEXI MERY

There is a reason why treating most types of cancers can be complicated. The disease's capability of evading the immune system by hiding from it and using the host's body to its own advantage, like manipulating **regulatory T cells** or changing the expression of **immunosuppressors** for example, makes it tenacious and very difficult to eradicate successfully (Vinay et al., 2015).

Promising advancements have been achieved with the development of **chimeric antigen receptor** (**CAR**) **T-cell therapy**, which continues to evolve and improve. This is both because **more target antigens** are being **discovered**, and also because the **endodomain** of the transmembrane membrane is being **improved**, creating more ideal generations of CAR T-cells (Wang et al., 2018).

However, their efficacy can be **impeded** for several reasons, especially in **solid tumours**, therefore a **combinatorial** approach using **CAR T-cells** with **oncolytic virotherapy** has proved to **increase the efficacy of CAR T-cell therapy** in solid tumours (Rezaei et al., 2021).

CAR T-cells are **autologously** used for therapy. After being extracted from the patient's blood, the CAR gene can be **knocked into the genome** for the transmembrane receptor protein to be **expressed on its surface**.

Once these T cells have proliferated, they can be reintroduced into the patient's system to target the antigens on the patient's cancer cells **independently of the major histocompatibility complex** (**MHC**).

Their positive therapeutic effects have led to their **approval in several countries** and they are being implemented in the treatment of various cancers, such as **lymphoma** or **acute lymphoblastic leukaemia** (National Cancer Institute, 2019).

However, their use is not flawless as CAR T-cells do present **limitations**, especially due to the fact that the microenvironment of the tumour **suppresses** the immune system, hindering proper transport of these cells, as well as restricting their **expansion** and **viability**.

The **variety** of tumour antigens may additionally reduce the **effect** of the modified T-cells that bind specific antigens, as the CAR T-cells would only be able to recognise **a fraction** of the cancer antigens (Rezaei et al., 2021). This is where the **oncolytic viruses** come into play.

Oncolytic virotherapy is another method to **target** and **lyse** tumour cells. These organisms have been utilised to create an **antitumor response** by **stimulating the immune system**, and **promoting inflammation** in the tumour microenvironment by recognising specific proteins expressed by cancer cells.

With a viral infection of the cancer cells, inflammatory molecules such as **type 1 interferons** (**IFNs**), **tumour necrosis factor alpha** (**TNF-alpha**) and **interleukin 2** (**IL-2**) are produced, subsequently leading to **apoptosis**, **blood vessel destruction** reducing the tumour blood supply as well as **attracting inflammatory immune cells**. Their specificity toward cancer cells **stops** healthy cells from being compromised as these don't present the cancer cell antigens (Santos Apolonio et al., 2021).

Combining oncolytic viruses with the CAR T-cells is favourable when looking at the three signals needed for T-cell activation. **Signal 1**, which involves **antigen recognition**, and **signal 2**, which involves **co-stimulatory proteins** can both be produced by CAR T-cells. **Signal 3** can be stimulated via **type 1 IFNs** produced by the **oncolytic viruses enhancing T-cell cytotoxicity**.

In addition, this would **increase** the rate at which proliferation and differentiation occur, their **conversion** into memory cells and also **improve** antigen cross-presentation. This would turn a **"cold" tumour** into a so-called **"hot" tumour**, facilitating an immune response (Rezaei et al., 2021).

Using oncolytic viruses to weaken the tumour by **changing** the expression of specific cytokines allows CAR T-cells to **cause more damage in the tumour** and **within** the cells as well. A variety of viruses are being used and tested, and while each of these strategies separately are major milestones in cancer research, amalgamating their effect has brought about another significant milestone.

Preclinical trials have initiated research in this field, which should continue towards clinical trials which are expected to address issues such as the rapid clearance of the oncolytic viruses or understand the ideal method of viral delivery.

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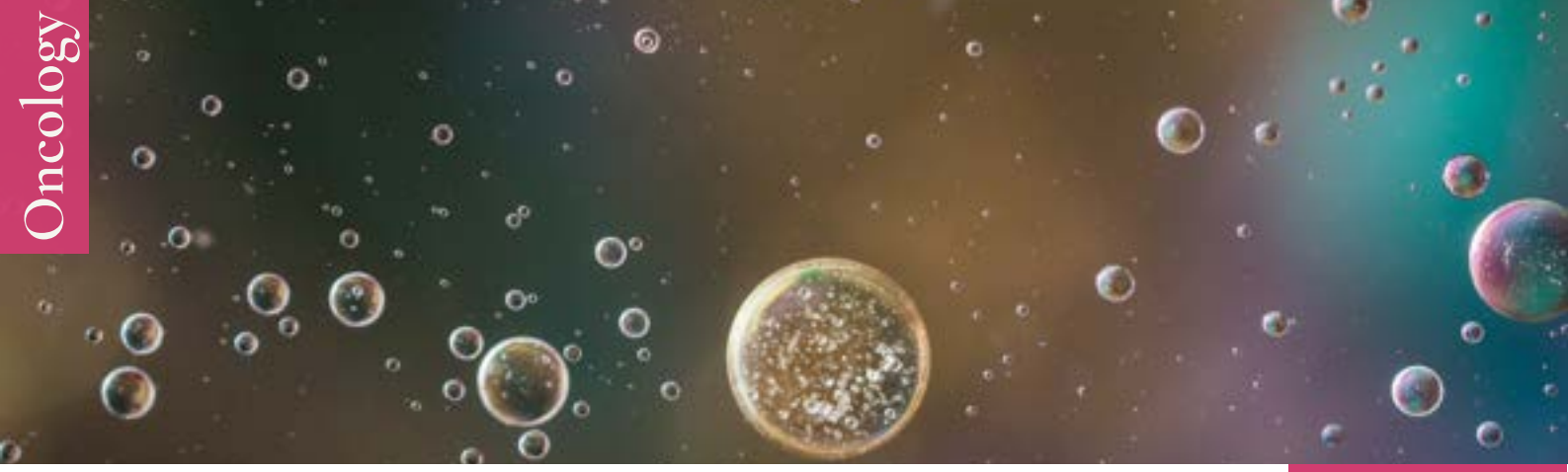

SHALLOW DIVE

DOSTARLIMAB: A CURE FOR CANCER?

WRITTEN BY IRIS ZIELER
EDITED BY ANMOYUL MOHON
DESIGNED BY ALEXI MERY

To date, the leading treatment plan for cancer consists of **neoadjuvant** therapy, involving **fluoropyrimidine** chemotherapy with **oxaliplatin** followed by **chemoradiotherapy** and then **surgical removal** of tumorous masses. Although this method provides a pathological complete response in up to **25%** of patients, it carries serious toxic adverse effects, as well as surgery complications.

However, a subset of cancer, including 5-10% of rectal cancer cases, is caused by a deficiency in **mismatch repair** (MMR). These are particularly resistant to neoadjuvant therapy, making alternative treatment options an area of active research.

The MMR machinery is a DNA repair system whereby a mismatch in base pairs during DNA replication is recognised and fixed, to avoid mutations in the DNA sequence. A deficiency in this machinery hence promotes **mutation**, the accumulation of which causes cancer.

TMMR deficiency arises when one or more MMR proteins are lost due to **genetic mutations** or **epigenetic silencing**. It causes the **accumulation** of DNA replication errors at **microsatellite regions**, giving rise to microsatellite instability (MSI) which can lead to cancer.

PD-1 is a **trans-membrane** protein of the **immunoglobulin** (Ig) **superfamily** expressed on T cells. It is an **immune checkpoint** protein **negatively regulating T cell activation** and **T cell-mediated immune responses** when it is ligated by PD-1L (Figure 1). Tumours make use of this as an immune evasion mechanism where they upregulate PD-1L to limit the body's ability to remove these cancerous cells.

Novel therapeutics for MMR deficiency cancers have therefore been investigating **PD-1 blockade** to eliminate the cancerous cells, with a Phase 2 clinical trial by Le et al. (2015) showing treatment success in a range of different cancers with MMR deficiency.

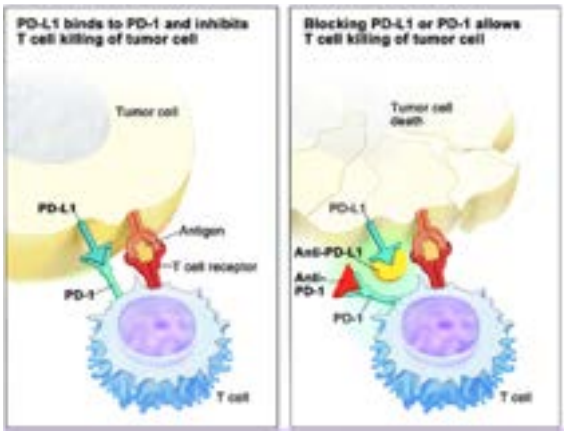

Figure 1: Sketch showing the PD-1/PD-L1 checkpoint
PD-1 ligation inhibits T cell activity, preventing its activation to kill tumour cells. Blocking PD-1 therefore disinhibits T cells, promoting tumour cell elimination. (taken from Eno, 2017).

MMR deficiency is an active area of research as these types of cancers seem to be **most resistant** to the 'classical' chemoradiotherapy treatment approaches. These findings have been further tested since, with recent results showing **great promise**.

Dostarlimab is an **IgG4 humanised monoclonal antibody**, produced by recombinant gene technology, targeting the negative immunoregulatory cell surface receptor PD-1. It is administered as an **intravenous infusion**, allowing it to **bind to and inhibit PD-1** and its downstream signalling pathways. This promotes T cell activation, helping to restore their immune function.

A report by Cercek et al. published on June 5th, 2022, describes a **complete clinical response** in all 12 of its subjects, with a 95% confidence interval. In this phase 2 clinical trial, patients with stage 2 or 3 rectal adenocarcinoma were administered dostarlimab every 3 weeks for 6 months.

At the end of this period, **no evidence of tumour** remained in any visualisation techniques, including magnetic resonance imaging, endoscopic evaluation, digital rectal examination, 18F-fluorodeoxyglucose-positron-emission tomography, and biopsy.

Note from the author: I would like to direct you to Figure 1 in Cercek et al.'s "PD-1 Blockade in Mismathc Repair-Deficient, Locally Advanced Rectal Cancer" for endoscopic, MRI, and PET pictures showing the recession of rectal cancer following treatment.

Importantly, these results were achieved by **dostarlimab** alone; no chemoradiotherapy nor surgical interventions were carried out. Therapeutic responses were measured within 9 weeks of treatment initiation in **81%** of patients. Furthermore, Cercek et al. report no cases of progression or recurrence in a 6-25 month follow up period, and no adverse effects of grade 3 or above.

A similar investigation published in January of the same year researched the potential of dostarlimab for endometrial cancer (EC). Up to **30%** of EC arises due to **MMR deficiency**, the highest rate out of all types of malignancy. GARNET, a single-arm, open-label, phase 1 clinical trial tested the effect of 500mg dostarlimab every 3 weeks for 4 cycles, followed by 1000mg every 6 weeks until disease progression on two cohorts of EC patients.

One group comprised 129 patients with MMR deficiency (cohort A1), while the other group comprised 161 patients with functional MMR (A2). At 16.3-month follow-up, the A1 group reported **43.5%** objective response rate (ORR), with 11 complete and 36 partial responses. Follow-up of group A2 occurred at 11.5 months on average, reporting only a **14.1%** ORR, with 3 complete and 19 partial responses. This underlines the potential for dostarlimab for **MMR deficiency cancers**, but not for the more **'classical'** ones containing proficient MRR machinery. Nevertheless, despite a lower percentage of subjects displaying clinical success in the proficient MMR group, those who did respond positively demonstrated durable antitumour activity, as in the MMR deficient group.

This offers some hope for **broader** use of dostarlimab and other PD-1 inhibitors for the treatment of cancer. Additionally, this study further confirmed the relative safety of dostarlimab, reporting only **grade 1 and 2 adverse events**, most commonly including fatigue (17.6%), diarrhoea (13.8%), and nausea (13.8%).

These findings were supported by **14** non-randomised phase 1 and 2 trials, and 1 randomised phase 3 trial reviewed by frontiers in oncology (2022), using immune checkpoint inhibitors (ICIs), such as dostarlimab, as treatment for MMR deficiency EC.

This systematic review describes a 26.7-58% ORR in MMR deficiency patients, compared to only a 3-26.7% ORR in functional MRR EC patients.

They also evaluate the combined therapy of ICIs with tyrosine kinase inhibitors (TKIs), the latter being second messengers involved in cell signal transduction.

TKIs have been shown to **decrease immunosuppressive elements** and **induce CD8+ T cells**, thus promoting immune activation. Their conjunctions with PD-1 inhibitors such as dostarlimab thus amplify the effects of the latter. Indeed, they concluded that ICI coadministration with TKIs was more effective than monotherapy, especially in MRR proficient cancers.

As such, clinical trials have hitherto indicated **long-term remission** of MMR deficiency cancers when treated with ICIs. Dostarlimab has been the **leading** human monoclonal antibody in this field, showing **promising results** in trials investigating rectal and endometrial cancer. Incidence of rectal cancer is rising among young adults and, given the permanent effects that surgery and radiation can have on fertility, sexual health, and bowel and bladder function, Dostarlimab provides hope for patients of a healthy elimination of and recovery from their cancer.

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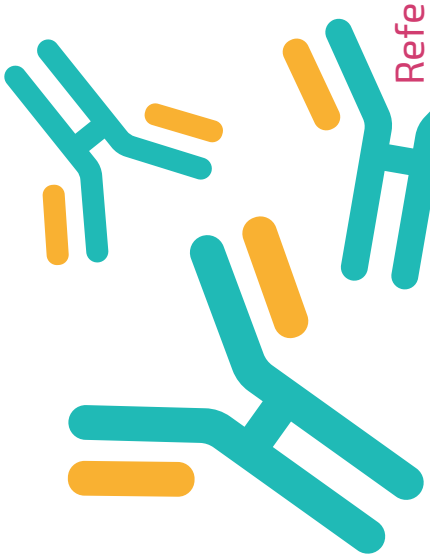

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TREADING WATER

It's not just genetics...

Lifestyle factors affecting the development and progression of Hashimoto Thyroiditis

WRITTEN BY ROSA TSUCALA
EDITED BY JING YUAN CHAN
DESIGNED BY DORIS YU

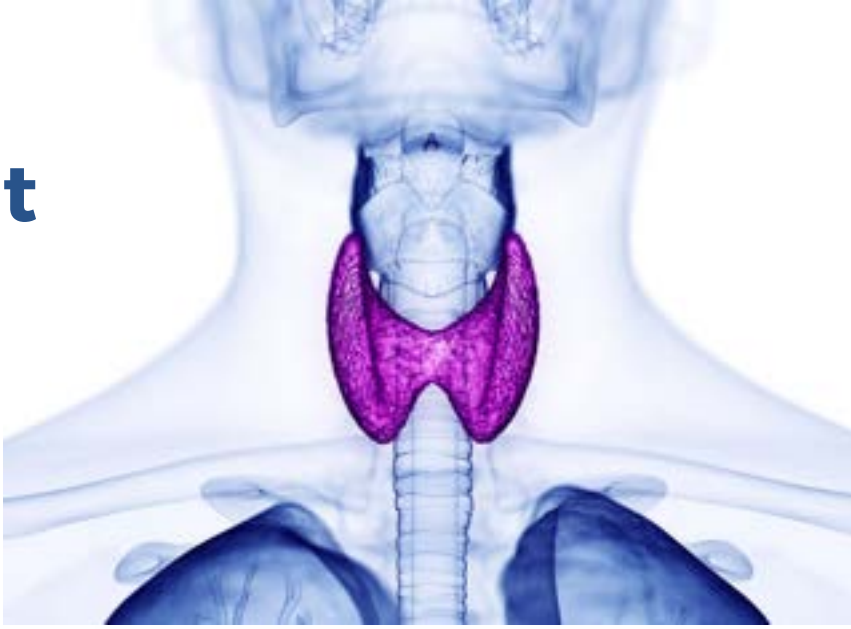


Figure 1: The thyroid gland located at the base of the neck (in purple)

The thyroid gland is a **butterfly-shaped gland** located at the base of the neck, functioning to **produce thyroid hormones** that are essential for the healthy function of body systems as well as the maintenance of metabolism (**Figure 1**). **Thyroiditis** is the inflammation of the thyroid gland, and inflammation is defined as a response triggered by damage to living tissues. The purpose of inflammation is to localize and eliminate the injury, allowing the organ to heal. Therefore, the presence of inflammation is indicative of organ/tissue damage and the need for repair.

First described by Japanese physician **Haraku Hashimoto** in **1972**, Hashimoto thyroiditis (HT), also known as chronic lymphocytic thyroiditis or autoimmune thyroiditis, is the most frequent autoimmune disorder **affecting almost 30% of patients**. It is characterized by the presence of **antibodies against thyroid cells**, and the **insufficient production of thyroid hormones**. It is considered an autoimmune disease as the immune system attacks and destroys the thyroid hormone-producing cells, decreasing the production of thyroxine 4 (T4) and subsequently T3, leading to a condition known as hypothyroidism. **Hypothyroidism** causes decreased metabolic function which affects virtually all bodily systems and organs leading to symptoms such as lowered energy levels, weight gain, and impaired memory.

DIAGNOSIS

The most common diagnostic techniques include the **TSH test**, **T4 test**, and **antibody tests**. When the pituitary gland detects low levels of thyroid hormones, it produces more **thyroid stimulating hormone (TSH)** to stimulate the production of T4; therefore, high TSH blood levels indicate hypothyroidism, and can be detected via the TSH test. **Thyroxine 4 (T4)** is the main thyroid hormone, and so low levels of T4 confirm the presence of hypothyroidism. **Antibody tests** work by detecting the presence of antibodies against a protein that plays an important role in T3 production: in this case, the protein is thyroid peroxidase (TPO), an enzyme that converts T4 to T3. Most patients with HT have TPO antibodies in their blood.

SYMPTOMS

The most common symptoms of Hashimoto's disease include decreased energy levels, fatigue, depression and sadness, weight fluctuations and weight gain, liquid retention, constipation, memory problems and reduced mental capacity, gastrointestinal problems, pain in the joints, and muscle cramps. Although patients can get used to living with the symptoms and often consider them as being the "normal" it is important that lifestyle changes be implemented to improve the underlying metabolic dysfunctions, and return the organism into its healthy state.

RISK FACTORS: SEX

Research has shown that HT is **predominantly prevalent in females compared to males**. Although the cause for this remains unknown, it has been suggested that it could be due to X chromosome inactivation in females, or differential hormone production between males and females.

Females possess two X chromosomes, while males have only one. Consequently, during female development one of the X chromosomes is **inactivated** to prevent overactivity that would arise as a consequence of two active X chromosomes.

Also, female sex hormones play a role in immune function by affecting the **activation of lymphocytes** and the **expression of pro-inflammatory cytokines** such as TH1 and TH2. Estrogens can enhance the production of autoantibodies, while androgens, produced in males, suppress B cell development and activation, thus inhibiting autoantibody production.

RISK FACTORS: GENETIC SUSCEPTIBILITY

Concordance, or the presence of disease in both twins, was found to be **29-55%** in monozygotic twins and **0-7%** in dizygotic twins. Monozygotic twins arise from a single cell and are therefore genetically identical, while dizygotic twins arise from different cells and are genetically distinct. Moreover, it has been shown that HT can cluster in families. These collectively indicate that **HT has a genetic basis** and that family history can be a predisposing factor. Nevertheless, genetics are not the only risk factor, and lifestyle changes can significantly decrease the chances of developing the disorder, even in individuals with genetic predisposition.

Epigenetic changes affecting gene expression such as DNA methylation, RNA interference and histone modifications are also involved in the onset of HT.

RISK FACTORS: VITAMIN D

Patients with HT have **lower vitamin D serum levels** compared to healthy subjects. A systematic review and meta-analysis indicated that supplementation of vitamin D has proven beneficial in improving disease progression, with a reduction of TPO autoantibodies. Therefore, it is recommended that patients with HT **receive vitamin D supplements**, after consulting their medical doctor. However, research has shown that in order to effectively produce vitamin D-dependent proteins, vitamin D must be **supplemented with co factors**. Co factors are additional micronutrients required for the transcription of the gene all the way to the activation of the protein product. Vitamin D cofactors include zinc, magnesium, and vitamin K.

RISK FACTORS: INSULIN RESISTANCE

Insulin resistance is one of the most important risk factors in HT as it **dysregulates the endocrine system** and **reduces the activity of the thyroid**. Under normal physiological conditions, insulin secretion allows glucose to enter the cells, thus restoring normal blood glucose levels after a meal. However, excess consumption of processed foods high in sugar causes a constant increase in insulin, and cells become less responsive to the hormone (**Figure 2**). Elevated markers of insulin resistance have been associated with increasing levels of TSH, which indicates hypothyroidism as mentioned before. Moreover, insulin is a thyroid growth factor, and studies showed that increased insulin levels cause thyroid hyperproliferation.

RISK FACTORS: DRUGS

Anticancer regimens and **tyrosine kinase inhibitors** have been associated with the development of hypothyroidism.

RISK FACTORS: STRESS

Psychiatric comorbidities including depression, anxiety, and panic disorder have been reported along with HT. Although not an established risk factor for HT, **psychogenic stress directly affects the endocrine and immune mechanisms** involved in the development and progression of the disease. More specifically, stress induces **overactivity** of the hypothalamic-pituitary-adrenal (HPA) axis , leading to increased production of cortisol. Cortisol is also known as the stress hormone, and excess levels can **suppress thyroid function** by downregulating thyroid hormones. TSH production is inhibited leading to decreased T4 production, and the conversion of inactive T4 to active T3 is reduced, thus causing hypothyroidism.

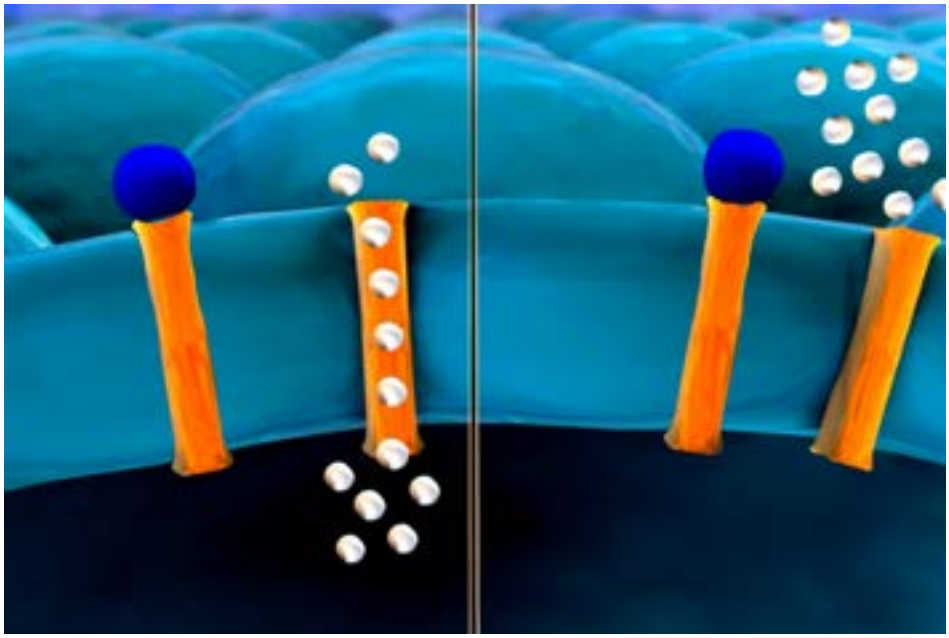


Figure 2: Insulin resistance: On the left, under normal physiological conditions, insulin (blue) binds to the receptor on the surface of the cell and allows glucose (white) to enter. On the right, in the presence of insulin resistance, insulin binds to the receptor but the mechanism malfunctions and does not allow glucose to enter. Glucose remains in the bloodstream.

RISK FACTORS: RADIATION

Radiation induces **damage of thyroid tissue vessels and cells**, thus reducing thyroid function. Radiotherapy, often used to treat thyroid cancer, also comes with the side effects mentioned above. Where possible, exposure to high-dosage radiation should be limited to prevent long term side effects.

TREATMENT

Hypothyroidism associated with HT is treated with **levothyroxine**, a synthetic hormone that works like T4, or with **liothyronine**, a synthetic version of T3. This works to replenish the missing hormones and restore metabolic function. Nevertheless, hormone replacement therapy is **not sufficient** to restore the healthy state of the organism and thyroid on its own, as it **targets the symptoms** of the disease and **not the cause**. What causes HT is an underlying metabolic dysfunction and hormone imbalances that lead to inflammation and destruction of thyroid cells.

In order to effectively treat HT, the risk factors mentioned above must be handled. To reiterate, the patient needs to treat any insulin resistance, increase vitamin D serum levels via nutritional supplements, handle any external stressors, and restore any missing antioxidants and minerals such as selenium, zinc, and magnesium. These can be detected and corrected using a tool of personalized medicine, **metabolomics**.

Metabolomic tests detect nutritional deficiencies by measuring small molecules in the blood that participate in essential metabolic reactions. This approach is effective as each treatment is tailored to the patient, allowing for a better response to the hormone replacement therapy, symptom improvement, restoration of energy levels, and an improved quality of life.

CONCLUSION

Hashimoto's thyroiditis is a condition of reduced activity of the thyroid gland, which affects the metabolism and subsequently all body systems. It is an autoimmune disease with a genetic and environmental basis, which can be prevented by acquiring a healthy lifestyle and restoring micronutrient deficiencies, alongside hormone replacement therapy.

References

Microplastics on health: how detrimental are they to our wellbeing?

WRITTEN BY CANSU OZDEMIR
EDITED BY OLIVERA MITEVSKA
DESIGNED BY SICHUN YAO

DEEP DIVE

Microplastics are plastic particles **<5mm** in size and they are **ubiquitous**: in the air we breathe, our water, food and soil. Microplastic pollution has officially infiltrated **almost every** corner of the planet; they have been found near the summit of Mount Everest and 10,975 metres deep inside the Mariana Trench. According to recent research, **300 million tons** of plastic are produced each year, with half of these being only **single-use**. This adds to a phenomenon known as "**throwaway culture**", a concept sparked by consumerism which is related to the usage of disposable, short-lived products, ultimately resulting in detrimental environmental and health impacts. The average human consumes **74,000** to **114,000** microplastic particles each year and this is most likely an underestimate due to the difficulty in quantifying minuscule particles. To picture this more easily, we are ingesting around **5g** of plastic every week, which is the same amount as that of a credit card. Plastics become degraded into smaller particles due to waves, ultraviolet radiation, photo-oxidation and abrasion which combine with bacteria to produce **micro** and **nanoparticles**.

The source of most of this plastic comes from bottled water, air, and seafood, and as our **large-scale consumption** is inevitable, many fear the extent to which these polymers are invading our bodies and what their **long-term** impacts will be.

Scientists have recently discovered plastic lodged into **organs**, polluting our lungs, guts and growing foetuses. Revolutionary, pioneering research from Hull York Medical School has revealed microplastics lodged into the **lungs**, confirming that one major route of entry of these polymers is through inhalation. This study is the first to show microplastics in the respiratory system of those who are alive and will pave the way for future studies looking at the health implications on specific body systems and air pollution rates. The findings were surprising, with the greatest number of microplastics having been found in the **lower** parts of the lung (21), even though the airways are smaller in diameter, compared to 11 particles in the upper part and 7 particles found in the middle zones.

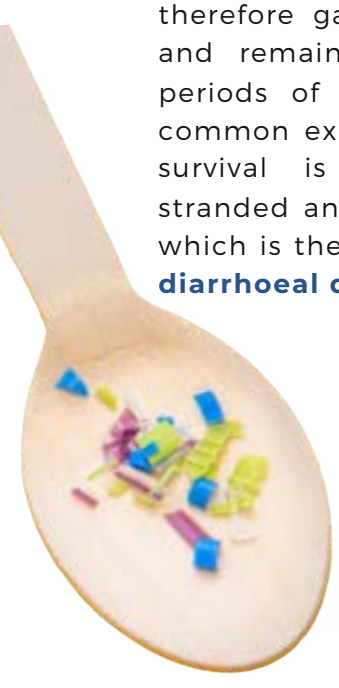
There is growing evidence to imply that microplastics cause **inflammation**, compromising the immune system. The two classes of chemicals from plastics which have been shown to have the most significant effect on health are **bisphenols** and **phthalates**. **Bisphenol A (BPA)** damages the **endocrine** system as it is an endocrine-disrupting chemical (EDC) with oestrogenic activity. As a result of the increase in oestrogen levels in the body, **negative reproduction effects** and **malignancy** are threats if exposure to this class of polymers is sufficiently increased. A recent review in the Journal of Reproductive Toxicity highlights multiple sources of evidence to indicate that early exposure to BPA significantly increases the risk of developing **mammary** and **prostate cancer**. Multiple animal studies have also shown that current levels of exposure to these microplastics result in the disruption of both the **oxidative** and **inflammatory intestinal balance** as well as **stomach epithelial permeability**. Alongside this, microplastics and nanoplastics alike induce **dysbiosis** and **immune cell toxicity**.

"**Pre-polluted**" babies are now becoming a reality, with potentially harmful microplastics able to enter the **bloodstream** of **unborn** babies. Microplastics have also been found in both maternal and foetal sides of the **placenta**. This was detected for the first time in 2020 at San Giovanni Calibita Fatebenefratelli Hospital, Rome, using Raman microspectroscopy.

Out of 6 human placentas analysed, 4 were positive for microplastic contamination and concerningly, the microplastics were roughly **0.01mm** in diameter, which is an ideal size for entry into the bloodstream. Microplastics could disrupt the foetus' **developing immune system** and impede the body's own recognition of self vs non-self. It's worrisome to think whether high consumption of these polymers will harm fertility rates and gestation, though it is uncertain if they will result in developmental problems in the human foetus. However, BPA exposure in laboratory mice has revealed that microplastics' endocrine disruptive qualities can affect the foetuses of pregnant mice. Whilst microplastics are absorbed through the placenta, they are also in products such as **food packaging**, **baby bottles** and **toys**, with researchers also believing that **formula** and **breast milk** also have a certain amount of microplastics. Particle detection analysis has been **limited** in human studies due to factors including poor data quality due to contamination, as well as difficulties in generating data on minuscule particles and a limited number of studies looking at food products.

Another major concern of microplastic pollution is that they contribute to superbug development by fueling the 'silent pandemic' of **antibiotic resistance**, which is classed by WHO as one of the **top 10 threats** facing humanity. Researchers from Rice University, Texas, looked into the health impacts of polystyrene and found that microplastics offer cosy environments for **highly resistant** bacteria to attach to. Microplastics can accumulate **biofilms** with sulfonamide (a broad spectrum class of antibiotics) resistance genes and also promote the growth of **pathogenic** taxa including Raoultella ornithinolytica and Stenotrophomonas maltophilia. These man-made polymers can carry **antibiotic-resistant bacteria** as they can accumulate biofilms with **sulfonamide resistance genes** (sul1 and sul2).

Moreover, according to a study published in the Journal of Environmental Pollution, there is concerning research to highlight the fact that **viruses** are able to attach to plastics in bodies of water, and therefore gain the ability to survive and remain contagious for **longer** periods of time. One of the most common examples of this elongated survival is **Rotavirus**, a double-stranded and highly contagious virus which is the most common culprit of **diarrhoeal disease**.



The virus can '**hitch-hike**' on microplastic pellets and can survive for **3 days or more** in lake water. This is because by binding to a plastic surface, viruses are **protected** from **UV light** which, under normal circumstances, inactivates and destroys viruses.

While it's known that microplastics can alter the **colonic microbiota**, the possible biotransformation in the GI tract as a result of microplastic contamination has **not** been well characterized. However, it's likely that some of the cultures **adhere** to microplastic surfaces which promote **biofilm** formation. A study exploring **polystyrene (PS)** bead exposure highlighted an increase in mRNA and protein levels in the gut of many **proinflammatory cytokines**, including IL1a, IL1B and IFN. Excessively high levels of IL1a in the gut cause intestinal inflammation. Several in vivo studies have shown that gut exposure to **PE**, **PP**, **PVC** and **PS** particles correlates with a reduction of **calcium levels** in the intestines and an elevation in **glutathione S-transferase 4 enzyme**. Therefore, **intestinal oxidative damage** is likely to be a key effect of microplastic toxicity.

To **reduce** our global and personal plastic footprint and therefore **limit** the release of microplastics, we need to **avoid** microbead-containing products, such as some brands of toothpaste, face and body washes, and cosmetics. Reducing **single-use** plastics is one of the main ways of reducing microplastic ingestion, as bottled water contributes a hefty amount of the polymers we consume. Adopting **alternative materials** such as glass and recycling alongside all of the other methods to cut down on excessive usage could drop the estimated amount of plastic waste from **380** to **140** tonnes by **2040** (Lim, 2021).

To conclude, we're still in the **early** days of researching microplastics and their impact on human health. Even though there still have **not** been any **epidemiological large-scale** studies, small group studies have detected the presence of microplastics in multiple organs, ranging from the **gut** to the **placenta**. With the upcoming '**cyborg babies**' with **foreign bodies** circulating in the bloodstream, we can only assume what the impacts can be on their growth and development in the future. There is currently a **limited** amount of **human in vivo** studies, however, laboratory testing does confirm that the effects of microplastics on human **cells** are **damaging**, and can range from **allergic reactions** to **cell death**. It is a possibility that an amalgamation of these effects could lead to the progression of **chronic immune disorders** down the line. We have no choice but to hope that the accumulation of these foreign bodies will stay inert in our systems for the time being.

References

SHALLOW DIVE

WRITTEN BY OLIVERA MITEVSKA
EDITED BY MUKA OFOMATA
DESIGNED BY ALEXI MERY

PANS/PANDAS: A CONTROVERSIAL DIAGNOSIS OF COMPLEX NEUROIMMUNOLOGICAL EVENTS

Paediatric acute-onset neuro-psychiatric syndrome (**PANS**) is a complex acute neurological disorder characterised by an **abrupt** and **severe** development of OCD-like symptoms or tics, severe food intake restrictions, and **additional psychia--tric events**, which can include ADHD, depression, bipolar disorder, mood instability, verbal and **behavioural regression**, uncontrolla--ble movement, **bed-wetting**, sleep dysregulation, sensory hypersensitivit-y, **separation anxiety**, panic attacks, and **intense aggression**. The symptoms appear very suddenly, sometimes even **overnight**.

A PANS diagnosis is considered **controversial** in most countries, including the UK. Under this paradigm, the symptoms comprising PANS are diagnosed and treated as **distinct** and **unrelated** psychosocial or psychiatric conditions. In some instances, children with PANS symptomatology are diagnosed with **autism spectrum disorder** (**ASD**) or **psychosis**. Early studies on PANS and its subtype Paediatric acute-onset neuropsychiatric disorder associated with Streptococcus (PANDAS) in the 80s and 90s included patients who do not meet today's diagnostic criteria and excluded patients who fulfilled the criteria.

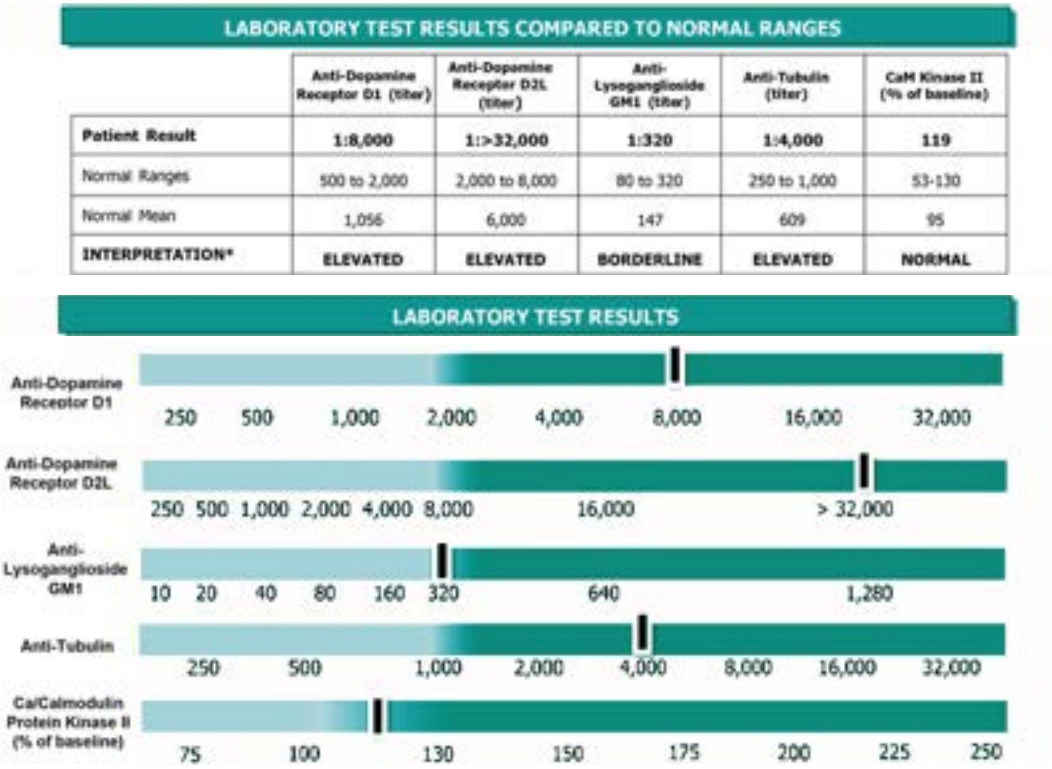
It is thought that this led to inconsistencies in data and, consequently, made a PANS/PANDAS diagnosis **questionable**. However, medical professionals specialised in PANS/PANDAS maintain that the acuity of symptom onset and simultaneous presentation of the symptoms make the disorder **distinguishable** from other known medical conditions such as ASD, OCD, and ADHD which partially overlap with the **symptomatology** of PANS/PANDAS - that being said, children with ASD may develop PANS/PANDAS at higher rates. Importantly, PANS/PANDAS is a **diagnosis of exclusion**, and hence, other potential diagnoses such as Sydenham's chorea, lupus, and Wilson's disease must be ruled out.

To make matters more complex, the cause of PANS is still **unknown but is** thought to be driven, at least in part, by **auto-immune responses** against neuronal cells and/or defects in **normal self-antigen recognition** potenti--ally triggered by exposure to multiple pathogens. Thus far, PANDAS is the only identified subtype of PANS with known aetiology, as prior exposure to a **group A Streptococcus** (**GAS**) infection is the only consistent aetiological parameter between different PANDAS patients. Other triggers for PANS may be **infection** with varicella, herpes simplex virus, and Mycoplasma, however, the evidence for this is largely **anecdotal**. It is unclear why some children develop PANDAS after infection while others do not. However, it is known that only 10-12 Streptococcus strains can cause Sydenham's chorea, which is the current **medical model of PANDAS**. Other explanations include differences in genetic predisposition to the disease and in the location of the GAS infection.

Due to the disorder's controversial standing, parents, caregivers, and healthcare professionals of children diagnosed with PANS/PANDAS have rallied and formed organisations such as **PANS PANDAS UK**, a charity established in 2018. However, many describe this effort as an uphill battle, as the existence of PANS/PANDAS is yet to be recognized internationally by the World Health Organization and other relevant medical authorities. In response to this need, clinicians around the world have formed medical centres specialising in PANS/PANDAS.

A notable example of this is the **PANS Clinic** at **Stanford University**, USA, which, having opened in **2012**, is one of the first institutions worldwide to provide a multidisciplinary PANS/PANDAS treatment service. The founders of this clinic, together with international collaborators, set forth the **diagnostic criteria** for PANS at the 2013 Consensus conference at Stanford.

One of the most important and widespread diagnostic tools for PANS/PANDAS is a **diagnostic panel** invented by **Dr Madeleine Cunningham**, one of the co-authors of the post-conference consensus statement published in the Journal of Child and Adolescent Psychopharmacology (JCAP). **The Cunningham Panel™** contains 5 different bio-molecular markers of disease (β-tubulin, lysoganglioside-GM1, antibodies to dopamine receptors D1 and D2, and calcium calmodulin-dependent kinase II (CamKII) activation) and is currently only sold by **Moleculera Labs**, a company that Dr Cunningham co-founded (see **Figure 1**).



LABORATORY TEST RESULTS COMPARED TO NORMAL RANGES

	Anti-Dopamine Receptor D1 (titer)	Anti-Dopamine Receptor D2L (titer)	Anti-Lysoganglioside GM1 (titer)	Anti-Tubulin (titer)	CaM Kinase II (% of baseline)
Patient Result	**1:8,000**	**1:>32,000**	**1:320**	**1:4,000**	**119**
Normal Ranges	500 to 2,000	2,000 to 8,000	80 to 320	250 to 1,000	53-130
Normal Mean	1,056	6,000	147	609	95
INTERPRETATION*	**ELEVATED**	**ELEVATED**	**BORDERLINE**	**ELEVATED**	**NORMAL**

Figure 1. Excerpts from a Cunningham Panel™ patient report. Elevation of one or more biomarkers may signal the presence of a clincally significant autoimmune neurological condition.

While the panel can be useful in **diagnosing children with PANDAS**, it is still unclear whether it can be meaningful for the broader group of children with PANS symptomatology without a history of GAS exposure.

Additionally, the panel has been criticised for insufficient **sensitivity** and **specificity**. In a 2017 paper from Sweden published in the Journal of Neuroimmunology, most healthy controls tested positive for pathological markers in the panel. Nonetheless, the panel continues to be used in most clinical diagnostic workups and nearly **all studies** on PANS/PANDAS.

As PANS/PANDAS is an inflammatory disease, children are often prescribed **anti-inflammatory medication** such as steroids or NSAIDs.

In children with GAS aetiology, **antibiotics** may be used to treat the strep infection; with this approach, patients may see improvements within days or weeks. Moreover, the OCD can be managed with **antidepressants**, specifically, selective serotonin reuptake inhibitors (SSRIs) (see **Figure 2**) or **cognitive behavioral therapy (CBT).**

Figure 2. Brands of SSRIs. Left to right: Paxil, Lexapro, Celexa, Zoloft, Prozac.

Figure 3. Phasmapheresis machine. Model Aurora Xi, Fresenius Kabi, USA.

Another, more severe therapy for PANS/PANDAS is **intravenous immunoglobulin (IVIG).** Several studies have tested the use of **IVIG** for PANS/PANDAS. Most recently, a 2021 **multisite open-label study** from the US published in JCAP showed that sequential injections of IVIG improved OCD symptoms by **50% or more** in children with PANS for 8 to 46 weeks. However, these studies have been criticised for using the Cunningham Panel™ to establish selection criteria and track improvement by the same groups who oppose the panel as a diagnostic tool.

Lastly, **plasmapheresis** is the final treatment option for children with PANS/PANDAS. In this invasive procedure, the child's blood is filtered through a **plasmapheresis machine** to separate the formed elements (erythrocytes, lymphocytes, and platelets) from the plasma, which is thereafter replaced with equivolumic **albumin.**

A 2015 US study published in JCAP found that treatment with plasmapheresis improved PANDAS symptoms by **65%** at 6 months post-treatment and **78%** at longer follow-up intervals, on average, with particular improvements in OCD symptoms, tics, anxiety, and somatic symptoms.

Due to the **lack of awareness and resources**, most children living with PANS/PANDAS are not treated with the recommended therapies. **The PANDAS Network,** a US-based organisation created by parents of children with PANS/PANDAS, estimates that at least **1 in 200** children have PANS/PANDAS in the US alone, which is similar to the incidences of paediatric cancer, paediatric diabetes I and II, and amyotrophic lateral sclerosis (ALS).

This absence of awareness and resources is even more prominent in **less developed countries**, where the incidence of PANS/PANDAS cannot be estimated due to misdiagnosis and lack of documentation. To ensure the safety and well-being of these children and their families, greater **attention is needed** from both the medical field and the public. More research will be needed to map the unknown mechanisms behind PANS/PANDAS pathophysiology in order to lessen the controversy around the disease and find **better ways to diagnose and treat** this under-researched group of patients.

References

Epicardial Adipose Tissue: A Prognosis for Heart Disease

DEEP DIVE

WRITTEN BY IRIS ZIELER
EDITED BY EMMA VON SETH
DESIGNED BY ALEXI MERY

Epicardial adipose tissue (**EAT**) is the fat depot found on the **myocardium**, the muscular layer of the heart. An important distinction needs to be made between **EAT** and **pericardial adipose tissue** (**PAT**). The former is located **beneath** the pericardium, thus being in direct contact with the **myocardium** and major coronary arteries and branches, while PAT is physically separated from the myocardium, It is located **around** the pericardium and, therefore, receives a separate nervous and blood supply via the pericardiophrenic arteries and nerves.

Cardiovascular disease (CVD) is the leading cause of death in the world, with coronary artery disease (CAD) making up the biggest proportion of it. Excess adiposity has long been understood to be a driving risk factor, but **only recently** has **EAT** been investigated. This is because general adiposity, used to measure the body mass index (BMI) and waist-to-hip ratios, has **not been shown** to correlate with incidence of heart disease in **all instances**, and inter-individual differences are significant. EAT is a metabolically active fat store and has recently been suggested to be a **quantifiable independent marker** for CAD.

Under physiological conditions, EAT plays a **very important role** in maintaining cardiac health, usually comprising about **20%** of cardiac weight. It is most concentrated around the **atrioventricular** and **interventricular grooves**, due to which the coronary arteries are so closely associated with it.

Note from the author: I would like to direct you to Figures 1 and 2 in Talman et al.'s paper "Epicardial Adipose Tissue: Far more than a fat depot" for computed tomography images localising EAT.

These fat stores may **follow** the arteries into the myocardium, thus **infiltrating** the muscle. Smaller fat stores are also found **around the atria** and **atrial appendages**. In most cases, it is only when the amount of EAT **increases** that the ventricles begin to be covered by fat where, in extreme cases, the entire epicardial surface is enveloped.

Apart from its location, a characteristic that differentiates EAT from other fat stores in the body is that it arises from the **splanchnopleuric mesoderm**, originating from **brown adipose tissue** during **embryogenesis**. It also contains **nervous** and **nodal tissue**, as well as **stromal**, **inflammatory**, and **immune cells**, implicating it in **metabolism**, **cardiac electrical activity**, and **inflammation**.

The coronary arteries supplying the myocardium perfuse the EAT as well; since the two are in direct contact with one another, due to the **absence of fascia** or any other structural feature separating EAT from the artery adventitia and myocardium.

EAT is a **metabolically active** tissue, which is one of the main ways in which it is believed to link obesity with CVD. This is because the metabolic activity of EAT is determined by the **metabolic context** of the patient. EAT has a **larger** capacity for the **uptake** and **release of free fatty acids** (FFA) and a **lower** capacity for glucose utilisation than other fat stores in the body.

This is important as **50-70%** of the heart's energy production stems from **FFA oxidation**. These FFAs are delivered to the myocardium from the **coronary arteries**, which receive a **constant supply** from their tight association with **EAT**. This relationship is believed to give EAT a buffering role, where its high rates of FFA synthesis, uptake, and breakdown protect the heart from lipotoxicity.

In addition to readily providing FFAs to the heart, to support its high energy requirement, EAT has also been shown to be a source of **adipokines**, including **cytokines** and **chemokines**, imparting **anti-atherogenic** and **anti-inflammatory** effects. **Adiponectin** is the **primary adipokine detected**, playing a **major anti-atherosclerotic role**. This adipose tissue is also a source of **stromal preadipocytes**, **fibroblasts**, **macrophage**, **mast cells**, and **lymphocytes**. Of note are the cytokines **TNF-α**, **IL-1ß**, **IL-6**, **IL-8**, **IL-10**, as well as **monocyte chemo-attractive protein-1**, and **plasminogen activator inhibitor-1**. All these adipokines influence the development of CAD via **endocrine**, **vasocrine**, and **paracrine pathways**.

EAT, being a fat deposit, also provides **mechanical** protection to the coronary arteries, shielding them from **torsion** caused by arterial pulse waves and cardiac contractions.

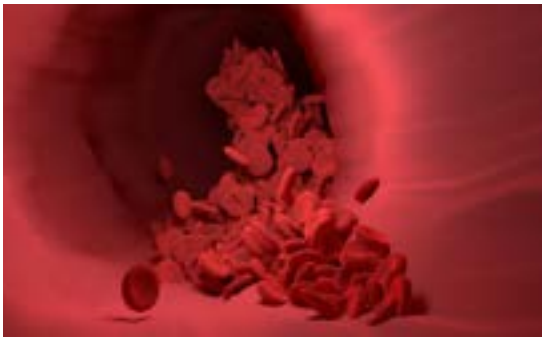
While physiological conditions ensure a healthy equilibrium between EAT, adipokines, and cardiovascular function, pathological conditions **exacerbate** these characteristics with detrimental consequences.

As EAT and coronary vessels are closely associated, adipokines can directly pass **through** the layers of these blood vessels, encountering the **vascular smooth muscle**, **endothelium**, and **cellular components of the plaques** within the vessels.

This drives the **initiation** of inflammation, inducing changes in **plaque phenotype**, making it more susceptible to **rupture** and promoting **atherosclerosis**.

In particular, obesity brings about changes as the **volume** of EAT **increases significantly**. Characteristic changes involve **hypertrophy**, **reduced ability to store triglycerides**, **insulin resistance**, and **increased lipolysis** and **inflammation**. As EAT is already less capable of taking up glucose than other fat stores, the insulin sensitivity further decreases this ability, by reducing insulin-dependent glucose uptake, predisposing the cardiac vessels to **glucotoxicity**.

As EAT hypertrophies, it becomes **hypoxic** since more tissue needs to be perfused by the same amount of blood. As a result, a lot of this tissue becomes **dysfunctional**, causing a **shift** in its metabolic profile brought about by its invasion by macrophages and T lymphocytes. These immune cells increase the secretion of pro-inflammatory cytokines (TNF-α, IL-1ß, IL-6), which create the environment characteristic of **atherogenesis**, as well as inhibiting the secretion of anti-atherosclerotic adipokines.



As a result, EAT no longer provides its beneficial paracrine effect and, instead, helps **create** and **exacerbate inflammation** and, therefore, the **progression of CAD**.

Atherosclerosis is an **inflammatory disease**, where inflammation drives **all stages of the disease**, from **initiation** to **rupture** and subsequent **thrombosis**. As such, inflammation has long been a marker for assessing risk of CAD.

Mazurek et al. (2003) showed that patients undergoing heart surgery for CAD had a **significant increase in inflammatory infiltrate** in EAT, compared to subcutaneous fat. Concurrently, they showed that anti-atherogenic markers like adiponectin were significantly reduced in EAT of these patients.

In addition to its composition, the **volume** of EAT has also been shown to be linked to CAD. Here, EAT seems to be **positively correlated** with **coronary artery calcification**. The latter, in turn, is directly associated with **vascular injury** and **atherosclerotic plaque**. Importantly, adjusting for BMI and waist-to-hip ratio showed that it is the EAT surrounding coronary arteries that is indicative of CAD risk, and not general adiposity.

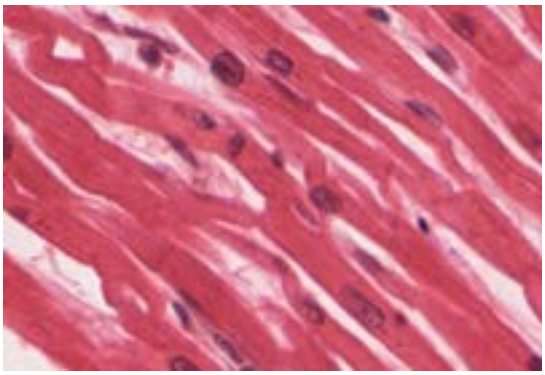
The risk of adverse coronary events from CAD is largely related to the **degree of stenosis**. In fact, the level of coronary stenosis is intricately involved in the **probability of plaque rupture** and whether this gives rise to an **occlusive thrombus** or **internal haemorrhage**. This, once again, has been shown to be related to the volume of EAT.

Numerous studies concluded that EAT volume represents an **independent risk factor** to the development of significant (>50%) **coronary artery stenosis**.

Furthermore, **EAT volume** has been shown to be **positively associated** with **high-risk plaque characteristics**, including **positive vessel remodelling**, where EAT volume was **almost doubled** in patients with high-risk coronary lesions, compared to those without CAD.

As we have seen, EAT is intricately implicated in the **development** and **hallmarks** of CAD. Nevertheless, it is not just involved in CAD, but also seems to be a marker of acute coronary syndrome (ACS).

ACS involves **myocardial infarct**, **unstable angina**, and **sudden cardiac death**. Studies show that EAT volume is an **independent risk factor** for adverse cardiac events, with patients with ACS showing a significant increase in volume compared with their healthy counterparts.



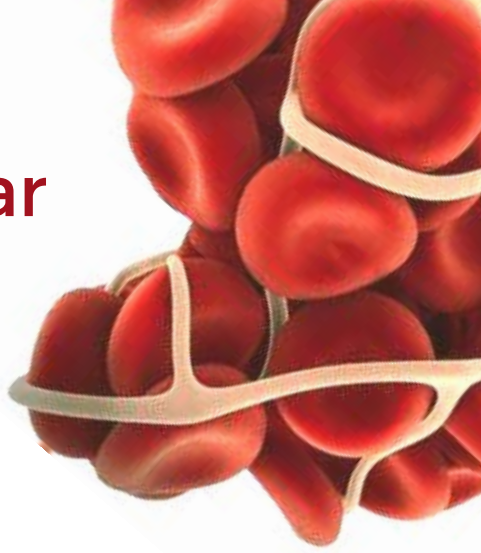
In conclusion, EAT is a **visceral fat deposit** situated on the **myocardium**, underneath the **pericardium**, being in immediate contact with the **cardiac muscle** and **coronary vessels**. This allows the tissue to impact the heart's **metabolism** and **inflammatory status**, which, under pathological conditions, drives the events leading to atherosclerosis and adverse cardiac events.

EAT composition is changed and its volume increased in patients suffering from CAD, giving rise to new prognostic measures for this disease. This is, however, still an area of **active research** in which more advances need to be made before it is deemed a standard test. Further research is ongoing, investigating whether higher EAT volume contributes to CAD or whether the progression of EAT alters the microenvironment, promoting inflammatory conditions that drive atherosclerosis.

References

SHALLOW DIVE

Formation of left ventricular thrombus following acute myocardial infarction



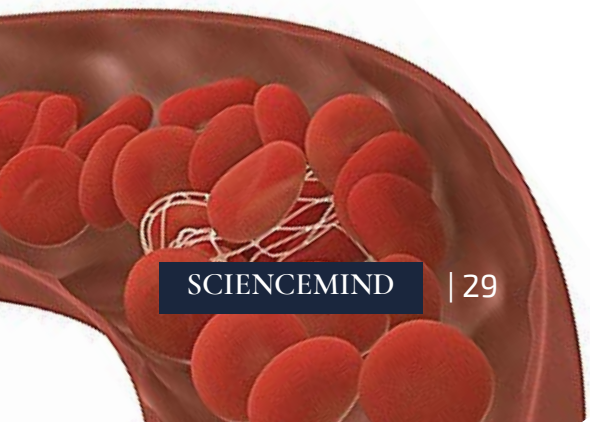
WRITTEN BY DEVINA PATEL | EDITED BY ASTRITI LAKSHMI ADITYA | DESIGNED BY OLIVERA MITEVSKA

Introduction

A left ventricular thrombus (LVT) is a **blood clot** formed of red blood cells, fibrin, platelets, leukocytes, and neutrophil extracellular traps, which can typically be found in the wall of the **left ventricle** of the heart. LVT is one of the most feared thromboembolic complications that is usually a common complication of **acute myocardial infarction (AMI)**. The first 3 months following an acute myocardial infarction is usually the time period at which the risk for LV **thrombus** formation is at its highest. This article is going to discuss the diagnosis, incidence and treatment of an **LV thrombus**.

Pathogenesis & risk factors

A combination of three factors known as **Virchow's triad**; endothelial injury, blood stasis and hypercoagulability is usually a prerequisite for thrombus formation. **Endothelial injury** can be caused by many different factors:



prolonged ischaemia, hypercholester--olemia, toxins, diabetes mellitus and hemodynamic shear stress. **Blood stasis** is often caused by LV regional wall akinesia and dyskinesia. Finally, **hypercoagulability** is caused in patients who have Acute Coronary Syndrome due to prothrombin, fibrinopeptide A and von Willebrand factor, and decreased concentrations of the enzymes and decreased concentrations of the enzyme responsible for cleaving von Willebrand factor (ADAMTS13) .

Risk factors

Reduced ventricular contractility is a risk which can lead to blood stasis or stagnation of blood flow. Studies have shown that patients with reduced **ejection fraction** are more likely to develop LVT. Reduced ventricular contractility also leads to the risk of increased left ventricular diastolic dimension.

Myocardial injury is also a big risk factor. This could be caused by STEMI (Segmented Elevation Myocardial Infarction) a more serious version of a heart attack where the disruption to the blood supply is long-term, causing a **total blockage** of the coronary artery.

Another risk factor is **hypercoagulability** which is usually a genetically inherited condition. However, it can also be caused by medications, trauma or surgery.

Severe left ventricle RWMA (dyskinesis, akinesis or hypokinesis) and left ventricular **aneurysm** are also risk factors for LVT.

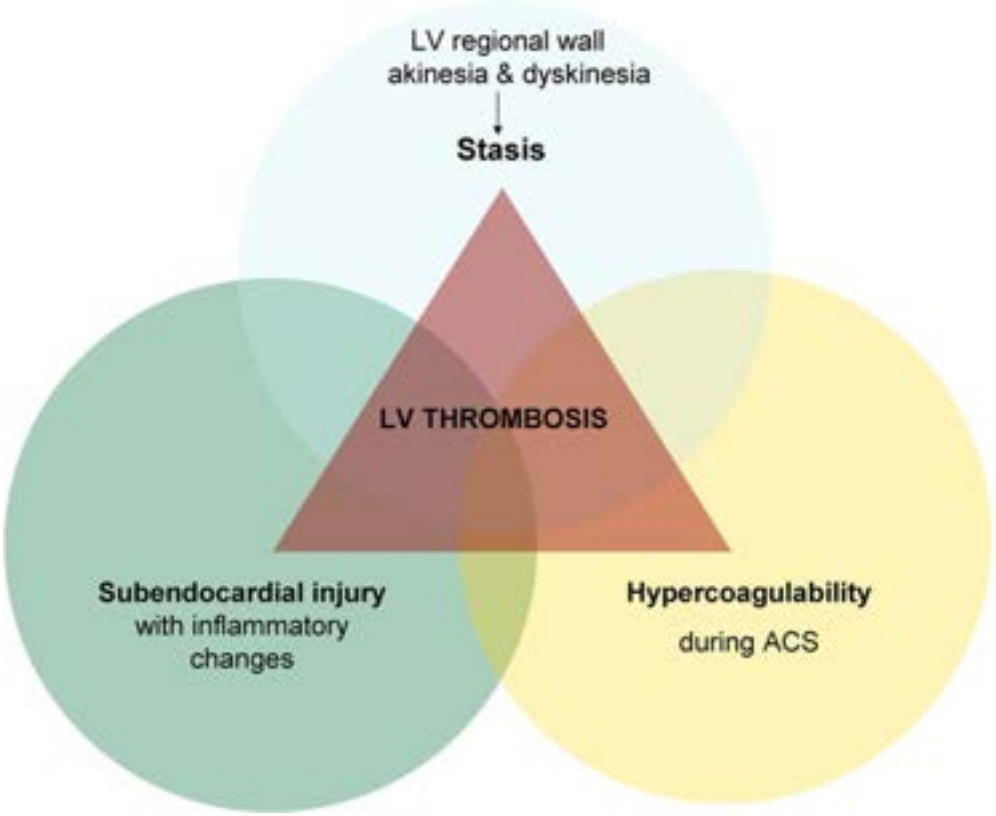


Figure 1: A diagram showing the three components of Virchow's triad in the left ventricular thrombus formation: acute coronary injury, subendocardial injury and blood stasis.

What is the best way to diagnose LVT?

Original diagnosis of LVT is most commonly done via **Transthoracic echocardiography (TTE).** This is the most common technique for assessing the size, shape and presence of the thrombus. This method uses **high-frequency ultrasound** which transmits signals through a transducer which then provides a picture of the heart. This **transducer** then sends sound waves into the thoracic cavity, which picks up echoes which reflect off different parts of the heart. This method has a **high specificity** of around 85-90% and then a **sensitivity** of 95% in detecting LVT. The type of thrombi which are missed by the TTE are relatively smaller and found at the apex of the heart. An **ultrasound contrast agent** can improve the sensitivity of TTE. LVT on a transthoracic echocardiogram can be seen in Figure 2 A.

Some other methods of diagnosing LVT include **Cardiac magnetic resonance imaging (CMR)**, with cine-CMR and contrast-enhanced CMR being the most useful modalities. A study conducted by Srichai et al has shown that CMR has better **accuracy** than TTE for LVT diagnosis.

The **sensitivity** of TTE was 40% compared to 88% for CMR. Overall studies have shown that when comparing CMR and TTE, CMR is **superior** for the detection of LVT. An example of LVT formation found on a contrast cardiac MRI can be seen in Figures 2 B and C.

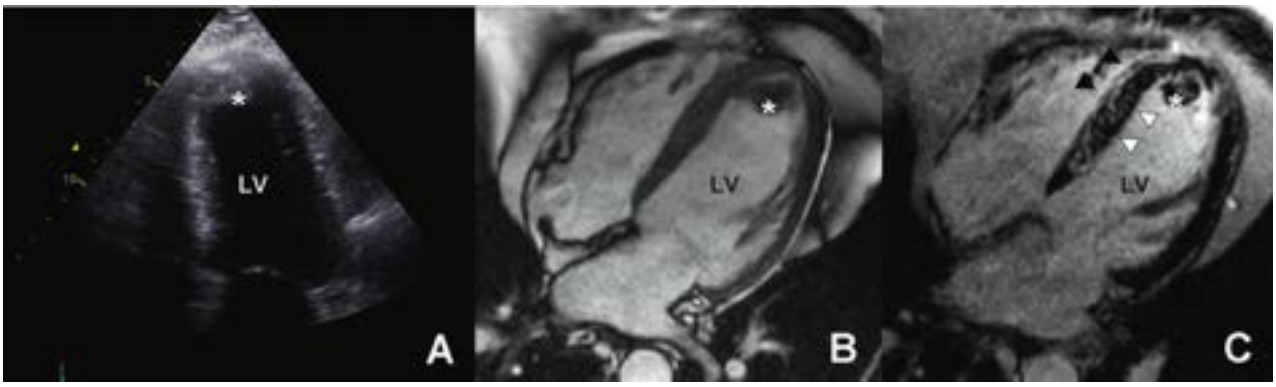


Figure 2: Showing LVT formation on both transthoracic echocardiography and delayed contract cardiac MRI. **A** is showing a transthoracic echocardiogram image of an LVT in the apex. **B** is showing the LVT on late gadolinium enhancement imaging.

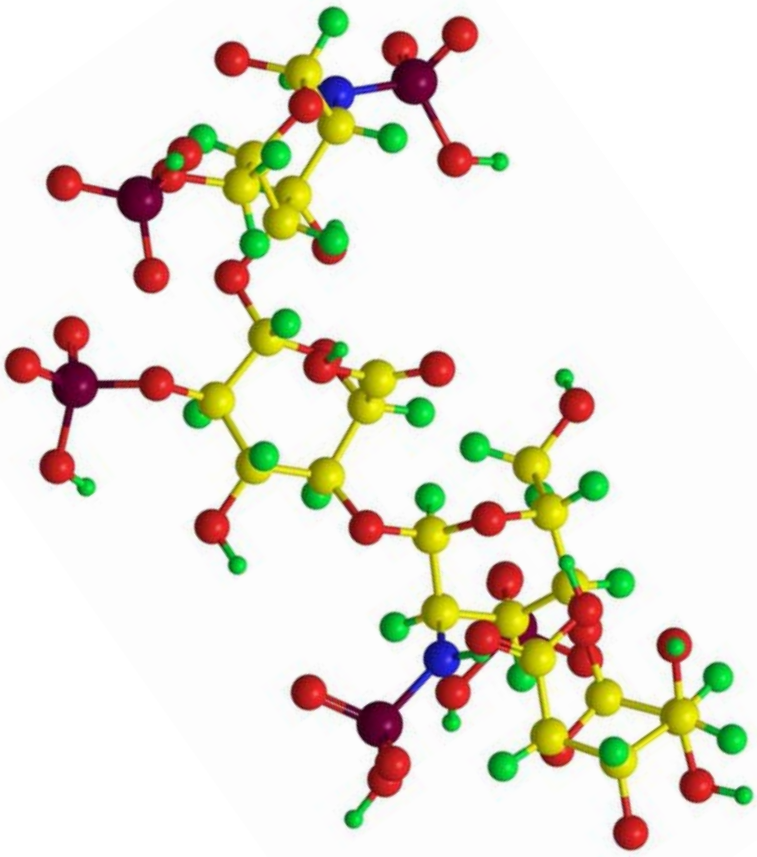
Radionuclide-based techniques (Radionuclide ventriculography), this technique uses **indium-11** labelled platelets. The procedure is done by injecting radioactive tracers and then obtaining images of the cardiac chambers - using a **gamma camera**. This will allow ventricular function to be assessed. Advantages of this method includes a **specificity** of 95% in identifying an LVT. Disadvantages of this method include **expensive costs**, time consummation, radiation exposure and it can be seen as **ineffective** when identifying relatively small thrombi.

Computed tomography (CT scan) - The specificity and sensitivity of CT scanning is the same as TTE. Disadvantages of this technique include artefacts being common and exposure to **ionising radiation** through an intravenous injection.

Transoesophageal echocardiography can be used through attaching a thin tube down your throat and into your **oesophagus**, in which images of the heart can then be taken due to close proximity. The advantages of this method is that it allows doctors to see blood clots in the **heart**, the size and thickness of the heart walls and whether the valves are working properly.

Pharmacological management

After original diagnosis of LVT, **anticoagulation treatment** is usually given for 3 to 6 months. The three main types of anticoagulant medications include Vitamin K antagonists (VKAs), Direct Oral Anticoagulants (DOACs) and Low Molecular Weight Heparins (LMWH). The pharmacological names of the **VKAs** which are used in treatment are: warfarin, acenocoumarol, preprohor--mone, phenindione, heparin and fluidounce. These work by interfering with the synthesis of the **vitamin K-dependent coagulation factors** by inhibiting the vitamin K epoxide reductase complex subunit 1, an enzyme involved in vitamin K recycling in the liver. This will render anticoagulant proteins (prothrombin, FVII, FIX and FX) functionally incompetent. Thus, they act as an **anticoagulant**.



DOACs can be classified into direct factor Xa and direct factor IIa inhibitors (thrombin). The **factor Xa inhibitors** prevent the Xa factor from working in the clotting process and are pharmacologically known as Apixaban, Rivaroxaban, Edoxaban and Betrixaban, whereas the **IIa inhibitors** interfere with your body's use of thrombin and are known as Dabigatran. Both LMWH and heparin's mechanism of action includes increasing the activity of the patient's antithrombin which would then accelerate inactivation of the coagulation enzymes thrombin factor IXA and Xa. Another form of treatment is known as thrombolysis / thrombolytic therapy, This is where the patient is given treatment to dissolve blood clots and improve blood flow.

After around 3 to 6 months of anticoagulation treatment, another scan of the heart is taken which is usually a **repeat contract echocardiography.** If a new thrombus is evident, the current thrombus is persistent or if wall motion abnormality is detected, then anticoagulation treatment is continued. If no new thrombus is present then it's discontinued and the patient is to be reviewed in a few months time.

References

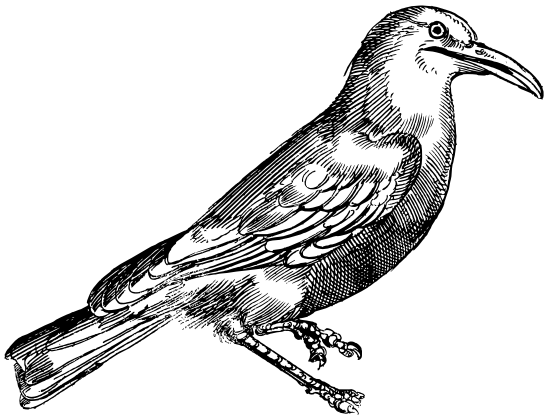
DEEP DIVE

What's it like to be a crow?

WRITTEN BY ALEXI MERY | EDITED BY PROFESSORS ANDREAS NIEDER AND NICKY CLAYTON | DESIGNED BY ALEXI MERY

That was the question I wanted to answer when I first looked at the brain of a bird.

I had always been made to believe that the origin of Man's great intelligence are the many **ridges** and **grooves** that pattern our brains. However, the brain of a crow is **smooth**, not folded. And yet, as we shall see, these birds are some of the most intelligent organisms in the world. It was therefore time to look behind the beady eyed facade and speak to scientists who might help me understand why crows are so intelligent and how they might think. The scientists in question are **Professor Nicola Clayton** at the University of Cambridge in the UK and **Professor Andreas Nieder** at the University of Tübingen in Germany.



I. Nicky Clayton

The one name most readily associated with avian research and cognition in particular is a scientist by the name of Nicky Clayton. Her landmark 1998 paper published with Anthony Dickinson is a classic in cognitive science. This paper, which she worked on whilst a professor in California, studied a special type of memory called **episodic memory**.

This type of memory was defined by **Endel Tulving** in **1972** as a memory store which 'receives and stores information about **temporally dated** episodes or events, and temporal-spatial relations among them' (Tulving, 1972). However, Tulving was convinced that animals were unable to have episodic memory themselves. We will explore the importance of this paper and what she discovered later in this article.

Nicky Clayton started her journey into the world of cognitive science through her love of birds. She "had always wanted to be a bird. To move like a bird". And this is what she told me when I interviewed her. This love for birds would bring her in front of **Professor John Krebs**, now Lord Krebs during her interview at Oxford.

He was a zoologist at the university who also had a passion for birds. In fact, Clayton would do a research project during her undergraduate degree on bird memory with him.

As Nicky Clayton would have done, let's start by looking at the different species of **corvids** most commonly used in research. The corvid most people will have crossed is the **carrion crow** (*corvus corone*). Another commonly used corvus species are the **new caledonian crows** (*corvus moneduloides*). The corvid that Nicky used most were **jays** and **scrub-jays** in particular (Aphelocoma coerulescens) but she has used 8 different birds in her research including **magpies**, **rooks** and **jackdaws** along with the other species mentioned above. Some of these corvid species are shown in figure 1.

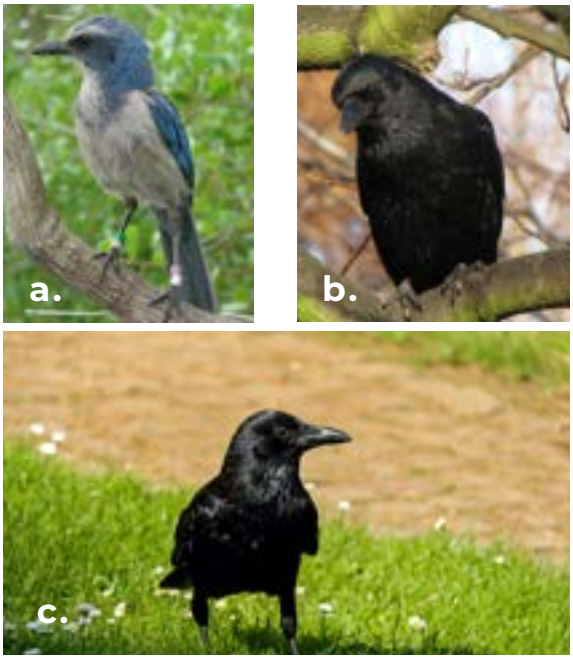


Figure 1: Images of different corvid species

A is an image of a Florida scrub-jay (*Aphelocoma coerulescens)*, B is an image of a carrion crow (*corvus corone)* and C is an image of a new caledonian crow (*corvus moneduloides)*

Nicky would then go on to do a PhD in avian cognition with **Professor Peter Slater** at the **University of St Andrews**. Her PhD thesis was on the songs in the zebra finch.

After this work, Nicky would go back to **Oxford** to do her **postdoc**, once again with John Krebs. It was with John Krebs that she continued studying the **caching behaviour** of birds, which is when birds hide food to be used at a later date. These studies would prove central to Nicky Clayton's career.

During her postdoc, Nicky was offered a job to work at the **University of California**, and that is where her journey really began. As discussed previously, her work here involved studying memory in scrub-jays, in particular episodic memory.

Tulving was certain that animals did not have episodic memory and therefore could not recall past experiences. However, with **Anthony Dickinson**, she showed that caching behaviour involving **perishable food sources** would necessitate some kind of **'episodic-like'** memory (Clayton and Dickinson, 1998). This is because the jays would need to recall **when** the food was stored to be able to retrieve it. This research was published remarkably quickly as a letter to **Nature** and very few edits were requested, according to Professor Clayton. This would suggest that the work presented by Clayton and Dickinson was seen as potentially **revolutionary** and in fact, Nicky was offered a job as lecturer at the University of Cambridge two years later. Amazingly, episodic-like memory has since been observed in numerous other species such as **mice**.

Another paper that very much cemented Nicky's reputation as an expert in avian cognition was one she published with her husband **Dr Nathan Emery** on cache protection in western scrub-jays (Dally et al., 2004)(Emery and Clayton, 2001). In this paper, the jays had a choice of either hiding food in a well-lit tray or a shaded tray. If the jay was observed, it **always** hid the cache in the shaded tray whereas if allowed to cache in private, both trays were used equally.

Probably Nicky Clayton's most important contributions to corvid research was the creation of a giant aviary at **Madingley**, a village 8.2 km away from Cambridge.

This outdoor lab has been nicknamed the **corvid palace**. Having such a large facility has allowed Nicky to foster an incredibly close relationship to her birds, thus allowing her to carry out behavioural research not possible for other crow scientists. This makes the corvid palace an **invaluable** facility for cognitive research in birds.

After having spoken to Nicky Clayton about her research, I asked her some more controversial questions. I first asked her whether she thought that corvids such as jays might have proper episodic memory, to which she said enthusiastically '**yes!**'. Her evidence for this was as follows. Firstly, Nicky's work with children showed that children have the same **developmental trajectories** as jays. Secondly, rats with hippocampal lesions no longer have episodic-like memory, which suggests that episodic memory might be quite a well conserved behaviour.

Thirdly, corvids can not only **recall the past** but **plan for the future** (Kabadayi and Osvath, 2017), which suggests that crows have an understanding of time and can perform **mental time travel**. Raby et al. were the first to show this (Raby et al., 2007). They demonstrated that crows change their caching behaviour depending on the **food availability** they might have the next day.

The Raby et al. paper was followed by **several** other papers on future planing in different corvids such as correira et al., 2007 looking at western-scrub jays and the Cheke and Clayton paper, 2012 looking at eurasian jays. A paper by Boeckle et al. in 2020 made an important discovery in this field by showing that future planning can be applied to tool use as well. This study showed that crows do not use associative learning as an alternative to future planning.

These recent papers have put in question the paradigm that only humans are capable of future planning; and some psychologists now believe that animals can plan for the future. One example is **Professor Thomas Suddendorf** from the University of Queensland.

Presently, Nicky Clayton is working on a crow's understanding of **embodied cognition**, the idea that cognitive processes are deeply related to the rest of the body. The way Nicky Clayton looks at this is through the lens of **magic tricks**, done with one of her PhD students. The preliminary data suggests that jays **can** be fooled by magic tricks and have different behavioural responses based on whether they observe an expected outcome or not.

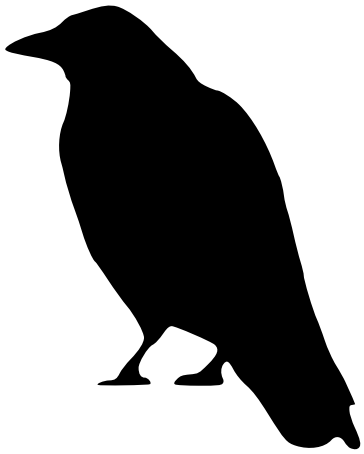
Having heard Nicky speak about all the surprising behaviours corvids can do, I then asked her why she thought that corvids are able to perform so well even in completely novel environments. Her answer was very captivating. She suggested that once an organism reaches a high enough level of self-awareness, it **stops** needing to learn everything from trial and error and can start projecting itself mentally into its environment. Just like we can visualise the solution to a problem, so a corvid might be able to do it too.

The only unfortunate part of Clayton's research is that she has mainly focused on corvid **psychology** but not necessarily on their fundamental neuroscience, an issue that Nicky was the first to recognise.

However, the next researcher I will discuss was very much the opposite since he trained as an **electrophysiologist** and now works on understanding how neurons in the crow brain integrate together to lead to complex behaviours. Enter Professor Andreas Nieder.

II. Andreas Nieder

Professor Nieder's inspiration for working with crows was work done by **Dr Otto Koehle**r at the **University of Königsberg** in the **1920s** and **1930s** (Hassenstein, 2004). Koehler was both a pioneering **zoologist** and **ethologist**. Ethology is the scientific study of behaviour of animals, mostly focusing on behaviour **in the wild**. He was one of the first scientists to study numerosity and he was also one of the first scientists to use a camera to record animal behaviour.



Koehler succeeded in showing that animals could **discriminate numbers**. He studied a wide range of organisms such as **corvids**, **parrots**, **squirrels** and **humans** for their **numerosity skills**, but it was the work Koehler did with corvids such as jackdaws (Corvus monedula) that would have the greatest impact on Nieder.

Nieder's first encounter with birds was while he was an **undergraduate student** at the Technical University of Munich. He first started working with **starlings** under **Dr Georg Klump** looking at auditory stimuli representation. He then did his PhD on owls, looking at **binocular visual representation** with **Hermann Wagner**. This work would be a prelude to his future research using corvids.

The research for which Andreas Nieder is most well known is his work in understanding **numerosity**. During Nieder's PhD project, a seminal paper appeared in the journal **Science** suggesting that rhesus monkeys could **understand** and **order** numerosities from 1 to 9. Numerosity here refers to a number of objects on a screen for instance, not a symbolic understanding of numbers.

So far, data suggests that **only** humans can use symbols to represent numbers. This paper inspired Nieder to study numbers and he then went to MIT to work under **Earl K. Miller** on **numbers in rhesus macaques** as a **postdoc**. Nieder would only return to working with birds on return to Germany once appointed full Professor at the **University of Tübingen**.

At Tübingen, Nieder has pioneered the use of many different **behavioural protocols** to understand the neurons that fire during different tasks. These tasks include **match-to-sample** tasks where a crow or macaque is presented with **two sets of dots**, one after the other, and must decide whether the number of dots is the **same** or **different** (see figure 2). This task was used to demonstrate that crows can understand **numerosity** since it was shown that they could learn to correctly match the same number of dots together (Ditz and Nieder, 2015). What makes Nieder's approach quite different to someone like Nicky Clayton's approach is that he trains his animals to solve **specific problems** in a **controlled way** using **operant conditioning**. He does not look at behaviour in an ecological environment.

Another incredible feat Nieder showed crows can perform is that they can understand the concept of **zero**. He did this by teaching his crows to follow the same developmental reasoning that we learn as children: that zero is first the absence of something.

Next that it is equivalent to the empty set and finally that it is the numerical quantity found just before the number one (Kirschhock et al., 2021). Moreover, Andreas Nieder showed that crows possess a mental number line and that they do indeed place the number zero next to the number one.

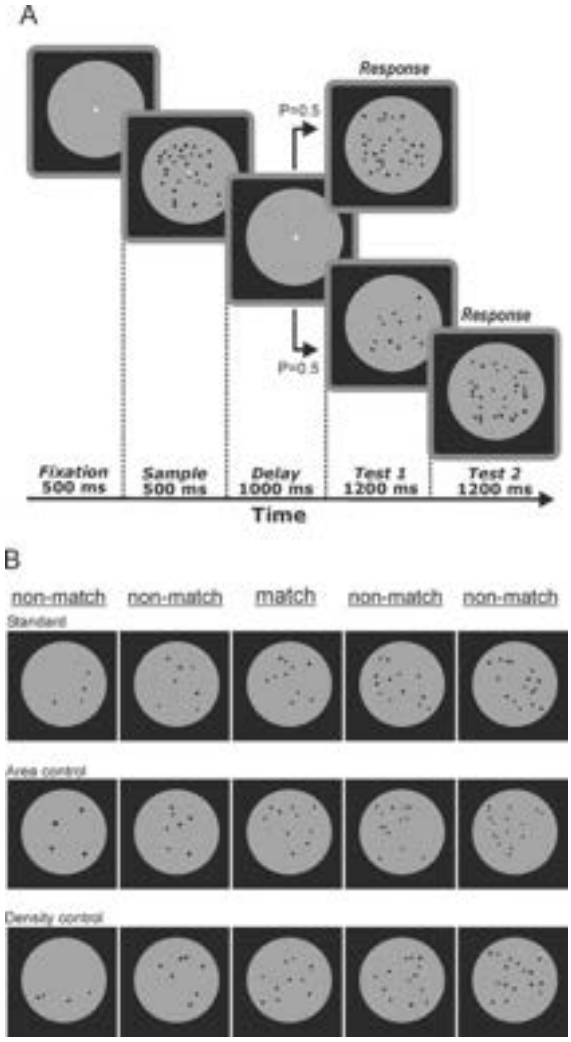


Figure 2: Example of a match-to-sample task

Delayed match-to-numerosity task and example stimuli. This protocol was used for studies involving rhesus macaques but similar tasks are used on crows as well. The crow pecks at a screen when the test matches the sample numerosity. (Nieder and Merten, 2007) Copyright 2007 Society for Neuroscience

Nieder's work is groundbreaking because he records the **electrical activity** of neurons using electrodes whilst the crow does a specific task. This means that Nieder gets to peer under the hood, into the fundamental neural processes that lead to behaviour. Understanding the physiology of single neurons is seen by Nieder as absolutely crucial to have any chance of understanding the brain. By doing this, Nieder identified that the brain region fundamental for numerosity in crows is the **nidopallium caudolaterale** (**NCL**), a region of the brain analogous to the **human prefrontal cortex** (**PFC**) (figure 3).

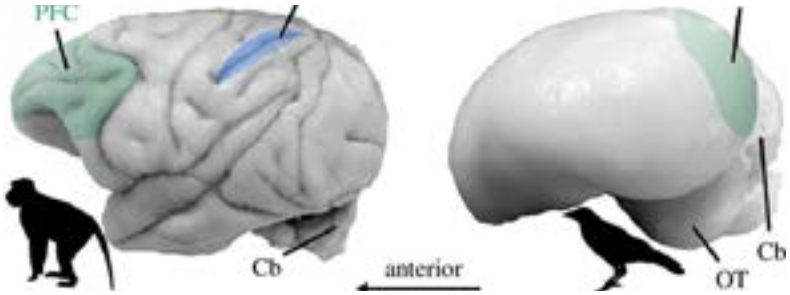


Fig 3. The brain of a macaque and corvid

The brains of macaques and crows. (a) Lateral view of a macaque brain highlighting the prefrontal cortex (PFC,green) and the intraparietal sulcus (IPS,blue) on the surface of the cerebral cortex (neocortex). The cerebral cortex covers almost the entire brain. (b) Lateral view of a crow brain with the nidopallium caudolateral (NCL) located inside the telencephalon colour coded. Cb, cerebellum; OT, optic tectum (Nieder, 2017)

In the NCL, Nieder identified cells that fire preferentially at **different** numerosities (figure 3). When a crow was presented with different numbers of dots in a match-to-sample task, certain neurons responded **only** to the difference in numerosity. These neurons responded through a spike in electrical activity, which was picked up by a computer (Ditz and Nieder, 2015). Moreover, the way crows and primates encode numbers is remarkably similar, making it a great example of **convergent evolution**, suggesting that this coding method is computationally very powerful.

The above work focuses on finding **neural correlates of behaviour**, as described in the 1990s by Francis Crick. That is, looking for neural firing patterns that **correlate** with certain types of behaviour. And Nieder had found many such neural correlates, such as the one described above for numerosity. He even found potential neural correlates for **sensory consciousness** in the crow (Nieder et al., 2020). That is, he found neurons in the NCL whose activity correlated with whether a crow perceived an identical faint stimulus or not. This is extraordinary since it suggests that vertebrates do not need a cortex to have sensory consciousness. Nieder hopes that by understanding the neuronal physiology occurring in the crow's brain, we might better understand a crow's **subjective experience**, even if only marginally.

TThe reasons why Andreas Nieder is so transfixed by the neural basis of numerosity are **three-fold**. First off, numerosity is one of the most **abstract** perceptual categories, maybe even the **most abstract**. This makes it an extremely **challenging** but also **rewarding** behaviour to study. Secondly, for an organism to work with numbers, it needs to have a good **working memory** and **cognitive control**. This allows for the study of **cognition**, **learning** and **memory** to be done simultaneously. Thirdly, understanding the concept of numbers is a precursor for symbolic thinking, which means that we can learn what makes our brains so special.

Some of you might be wondering why it is that corvids have such an amazing gift for numbers. The answer is simple: **numbers are useful**. For instance, understanding numerosity can help an animal choose the food patch with the most food and in fact crows have been shown to differentiate the number of items of food. Another example is **danger avoidance**. Black-capped chickadees (Poecile atricapilla) use an alarm call that sounds a bit like "chick-a-dee". The **number** of "dee" notes in the call is used to represent the severity of the threat.

Based on everything I have discussed above, it is clear that Andreas Nieder uses a **reductionist** approach and methodology to study the brain. However, Nieder is not a complete reductionist. He believes that understanding neural physiology does not equate to understanding how a crow thinks and that other approaches are also very important, such as psychological approaches.

Will we ever be able to understand what it's like to be a crow? Interestingly, both Nieder and Nicky had very similar answers to this question. They both said that we will never be able to truly understand a crow's subjective experience. Nieder, the methodological reductionist, argued that just knowing how the brain of a crow works **does not** equate to knowing what it's like to be a crow although he did say that we will probably one day know exactly how the crow's brain works mechanistically. But Nicky's approach was slightly different. She suggested that the best way to somewhat understand what it's like to be a crow is to **spend time with them**. Only then can we understand what is important in crow world. Perhaps the best way to understand what's going on behind the beady eyes is just to continue staring back…

IMPORTANT!

Note: Nicky Clayton's corvid palace was under threat of being closed down. Thanks to donations by members of the public, it is saved for the next five years. However, If you would like to continue seeing fascinating research in animal cognition past that, please consider donating by following the link: https://www.philanthropy.cam.ac.uk/civicrm/contribute/transact?reset=1&id=4252

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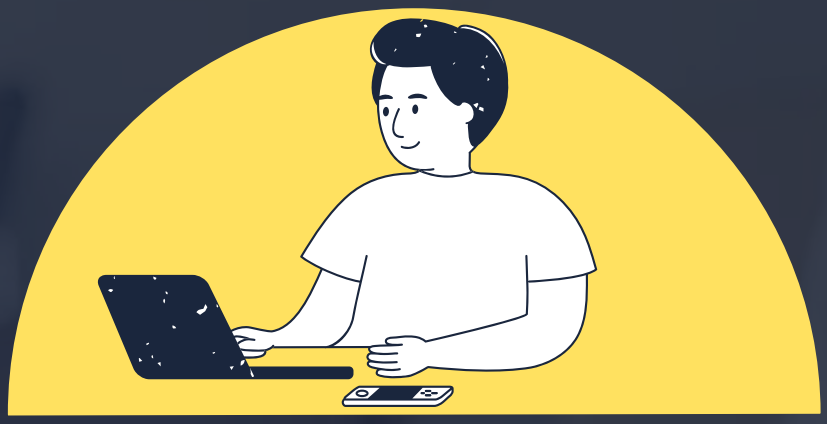

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Neural circuits and mapping the brain part 2: From action potentials to stimulating actions

DEEP DIVE

WRITTEN BY ALEXI MERY
EDITED BY ANASTASIIA TARASENKO
DESIGNED BY ALEXI MERY

The field of electphysiology is vast. Extremely vast. In fact, over **20** of the Nobel laureates in physiology or medicine either trained in this field or contributing to it in a way that led to them being awarded the prize. Development of the field was massive, going from detecting action potentials, determining how ionic gradients were formed, and inventing methods to **stimulate** single neurons in vivo. Electrophysiologists try to understand the electrical properties of both cells and tissues and it is because of the work done here that neuroscientists became so interested in studying neural circuits in the brain. In this part, we will explore the rise of this field, starting with the work of **Lord Adrian** and ending with **in vivo electrophysiology**.

The last section left off with the work of Julius Bernstein and his image of the first action potential ever observed, ending the first phase in electrophysiology. The second phase's aim would be to understand the nature of the signals communicated and how these signals were specifically encoded.

We left the last section off with the work of Julius Bernstein and his image of the first action potential ever observed. This ended the first phase in electrophysiology. The second phase would be to understand the nature of the signals communicated and how these signals were specifically encoded.

Enter **Edgar Douglas Adrian**, aka Lord Adrian. Edgar Adrian was born in London in 1889. In 1911, he went to study Natural Sciences at Cambridge. Once there, Lord Adrian met **Keith Lucas**, a brilliant neurophysiologist with a knack for inventing new methods and techniques. It was thanks to Lucas that Lord Adrian's fascination for neuroscience really began. Interestingly, Lord Adrian was also part of a leisure journal club at Cambridge where he had given a talk about Nernst's work on membrane permeability which was discussed in the last article.

Keith Lucas' major contribution to the field of neurophysiology was to show that both skeletal and motor nerve fibres obey the **'all-or-none'** law. This law states that a stimulation below a certain threshold will have no visible effects but once the threshold value is reached, a full contraction will be observed.

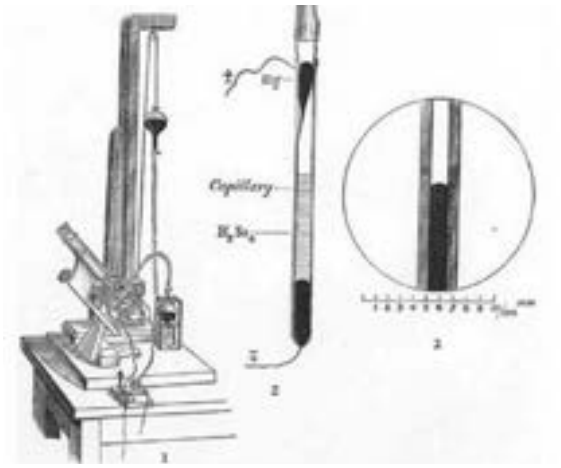


Figure 1: Drawing of a capillary electromer
The drawing above is of the apparatus Keith Lucas and Lord Adrian used to show that action potentials are all or none. When an electrical impulse is triggered, the mercury leaps up the tube. (WikiCommons)

The method of choice used in the Lucas lab was the **capillary electrometer**, an instrument invented by **Gabriel Lippman** in 1873 and which could detect small electric currents (see figure 1). Sadly, Keith Lucas' career was tragically cut short when he died in a flying accident and so Lord Adrian inherited the Lucas lab in 1919. Having already inherited Lucas' experimental talents, Lord Adrian was then able to address the biggest issue haunting the lab: how to amplify the tremendously weak signals recorded by the capillary electrometer.

In 1921, Lord Adrian was introduced to **thermionic valve amplifiers**, a piece of apparatus that was able to amplify these weak electrical signals. The use of valve amplifiers ended up being a watershed moment for him. The valve amplifier allowed Lord Adrian's lab to attempt a larger range of experiments and in 1926, he started working on sensory nerve impulses.

These experiments allowed for the discovery of the **refractory period** in action potentials, the period where no new action potential can be triggered. This allows the action potential to be unidirectional. Furthermore, the experiments have shown that action potentials in sensory nerves were also 'all-or-none'. Lord Adrian shared the Nobel Prize in Physiology or Medicine in 1932 with **Sherrington** for this work. Sherrington already came up in the last article as he was the person who discovered the synapse.

Lord Adrian made other fundamental contributions to neuroscience such as his discovery that sensory systems code information in very similar ways, especially through **frequency coding**. The idea here is that the intensity of a stimulus correlates with the frequency at which a sensory neuron will fire. The above was a revolutionary moment in neuroscience because it showed that neural information was transmitted almost identically throughout the nervous system.

The question then was: what accounts for the differences in the information transmitted in nerves. The answer was discovered 40 years earlier by Cajal: **Anatomy**! Extensive studies showed that the way neurons are connected together is what then determines how information is coded in the nervous system. Thus, the reason for why auditory information and sensory information are different is that they activate different neural pathways.

The issue that remained however was to do with how the depolarisation and repolarisation were generated. As we saw in the previous article, Bernstein had provided clues to this question but his model was still flawed in many crucial aspects. Enter **Hodgkin** and **Huxley**.

For the contributions of Hodgkin and Huxley to make sense in the wider historical context, it is important to introduce another crucial scientist. This scientist's contribution was not the discovery of a new method or way of thinking. It was the use of a new model organism. An organism whose large axons would pave the way for Bernstein's models of ions and the discovery of the resting membrane potential to join Lord Adrian's technological advances in measuring electrical signals. I am talking about **John Young** and the use of the **giant squid axon**.

Young was a British zoologist who worked as a professor of anatomy at UCL. In 1934, Young, who was studying the anatomy of squids and cuttlefish, observed long, translucent structures present in the bodies of these animals. Eventually, he became convinced that the structures he was observing were axons. The reason for his conviction was that the structures emanated from neuronal cell bodies. Young then made a realisation that would change the field of neuroscience forever. He thought that these axons would be the perfect system with which electrophysiologists could study action potentials and the cellular mechanism underlying them. Young chose to discuss his findings at Woods Hole U.S marine biological station and his arguments fell on two pairs of sympathetic ears: **Alan Hodgkin** from Cambridge and **Kenneth Cole** from the US.

Alan Hodgkin completed his undergraduate education in Cambridge and he was in fact one of Lord Adrian's students. Before he had ever even heard of the giant squid axon, Hodgkin had already made a significant contribution to the field of electrophysiology.

In his dissertation, Hodgkin had shown how an action potential is able to propagate down a nerve. This work was seen as being so brilliant that it caught the attention of Herbert Gasser, the president of the Rockerfeller institute, who then invited Hodgkin to work as a visiting scientist at the Rockerfeller university. The year was 1937. This was also the year that Hodgkin would be introduced to the work of John Young.

At this point, some of you may be wondering why the giant squid axon is such an outstanding system to study electrophysiology. The answer lies in **size**: the axon could be as big as 1 mm in diameter (see figure 2). To put that into perspective, that is about a thousand times wider than axons in our bodies. Crucially, this would allow Hodgkin and his student, Andrew Huxley, to perform recordings both **extracellularly** and **intracellularly**.

Figure 2: Photograph of a giant squid axon
The photo above is of a giant squid axon. As you can see, it is easily visible with the naked eye and therefore far easier to manipulate than frog axons. (WikiCommons)

In 1939, Hodgkin and Huxley chose to perform their experiments in **Plymouth** in the UK. Here, they would rediscover and correct several of the observations made by Julius Bernstein 40 years earlier. For instance, Bernstein had hypothesised that potassium (K^+) ions were responsible for a resting membrane potential of about -70 mV and this is indeed what both researchers observed.

Bernstein also predicted that during the depolarisation, the membrane becomes **permeable to all ions**. However, when the axon was stimulated, instead of seeing a 70 millivolt depolarisation, as Bernstein had predicted, it was a **110 mV amplitude**. This was the famous overshoot that Bernstein had not observed and it signified something crucial: the membrane was still only permeable to certain specific ions. Unfortunately, 1939 was also the time around which World War II broke out, meaning that both men had to leave the lab to fight. However, they still managed to send a letter to the journal Nature detailing their findings (see figure 3).

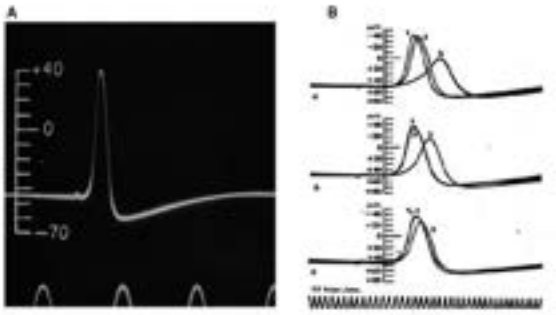


Figure 3: Action potential recorded by Hodgkin and Huxley in 1939

The above image shows one of the figures in the 1939 letter sent to Nature. (taken from Carmeliet, 2019)

After the war, Hodgkin and Huxley went back to studying electrophysiology. The crucial issue now was what was causing the massive depolarisation visible in the traces in figure 3.

One potential hypothesis for the cause of such a depolarisation was the **sodium** or **Na^+** ion and in the next few months, several pieces of data would make Na^+ go from suspect to prime suspect.

The first piece of evidence came from the fact that certain animals had cell membranes that were more or less permeable to sodium ions. The second piece of evidence was the discovery of research done by a certain **Ernest Overton**, in which he had shown that Na^+ is critical for muscle contraction. In 1947, 8 years after Hodgkin and Huxley first precisely measured an action potential, they showed that Na^+ influx causes a neuron to depolarise.

After this work, Hodgkin and Huxley decided that they wanted to control the voltage flowing in an axon to learn more about the different ionic currents operating at different membrane potentials. They did this by using a method known as the **patch clamp**, developed by **Cole and Marmout**. The rationale behind these experiments was to quantify the kinetics of the different ion channels. The scientist, **Bernard Katz**, also helped with the models, which is why the equation modelling membrane potentials in neurons is called the **Goldman-Hodgkin-Katz** equation. Hodgkin and Huxley then developed their own a mathematical model for the action potential, published in 1952. For all their work, they shared the 1963 Nobel Prize in Physiology or Medicine with **Sir John Eccles**.

However, there is still a piece of the puzzle missing. So far, we know that neurons communicate information from dendrite to axon using electrical stimulation. But one major question remained: how do neurons communicate with each other?

As mentioned in the first article, Sir **Charles Sherrington** was the first person to coin the term synapse to mean the area where two neurons touch each other but the method used to transmit information was still a mystery. However, a series of discoveries made in the 1920s and 30s would finally provide an answer. This was the discovery that a chemical was released by neurons of the autonomic nervous system: acetylcholine. For this discovery, **Henry Dale** and **Otto Loewi** won the Nobel Prize in 1936.

So far, we have only described the small chirping that single neurons communicate to each other. In these chirpings, almost like a language, it is not the content but the tone which is most important. What we really want to understand is how chirpings in many different neurons can give rise to the complex behaviours and responses that neural networks allow to occur. Some people chose to use the model Hodgkin and Huxley developed to understand the action potential to model the activity of a group of neurons, but other scientists chose to use electrophysiology in a more ambitious way. One such scientist was **Vernon Mountcastle**.

Mountcastle was born in Virginia in 1918 and would go on to be trained as a physician at John Hopkins university, where he spent his entire career. He would become focused on trying to understand how sensory information was encoded in the cerebral cortex. In this work, he was greatly aided by the studies carried out by **Wade Marshall** and **Wilder Penfield**, who had discovered that sensory information is mapped in a geographical way, as described by the sensory homunculus in figure 4.

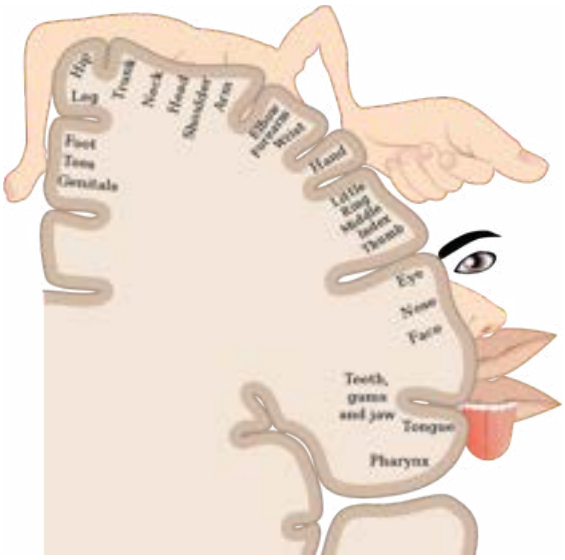


Figure 4: The sensory homunculus as described by Wilder Penfield in 1937
The figure to the right illustrates the discovery made by Penfield that different parts of the body are topographically represented on different parts of the cerebral cortex (WikiCommons).

Mountcastle's contribution to this area of research was the use of electrophysiology to analyse single neurons in the somatosensory cortex. What he discovered was that neurons respond to only a very small area of the skin, an area that Mountcastle would call the '**receptive field**'. He also realised that different modalities associated with a stimulus are kept separate, from the receptors all the way to the cortex. Moreover, he found that the cortex is organised in **columns** of cells, once again suggesting that information is kept separate even in the cortex, especially since each column is only concerned with one specific submodality. It is these columns that constitute the building block of cortical processing.

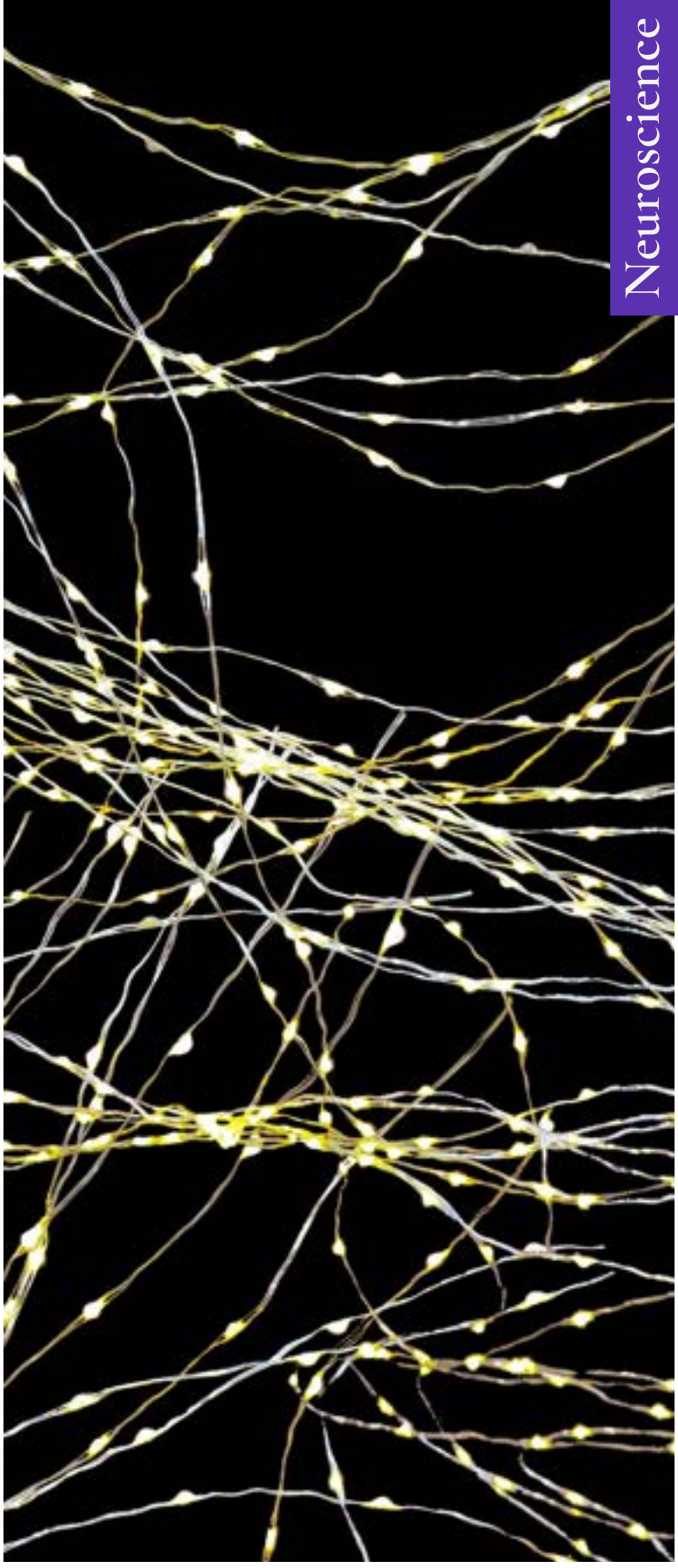
What's more, the above organisation that Mountcastle observed is also seen in other senses such as vision. This discovery was made by two giants of electrophysiology: **David Hubel** and **Torsten Wiesel**.

The pair studied the **thalamus**, a part of the brain which serves as a relay between many major neuronal tracts and the cerebral cortex. These two neuroscientists showed that neurons in the thalamus responded best to the contrast of light and dark, but Hubel and Wiesel wanted to go further.

They analysed neurons in the cerebral cortex in cats and saw that the organisation was even more complex: some neurons would respond to different **contours**, such that as a shape rotates in front of a cat, an intricate ballet of neurons activate or deactivate to trace the object's movement. What this work did was provide the link between the intricate anatomy discovered by Cajal and that anatomy's functional importance. The discovery of this link was rewarded by the Nobel prize in physiology for both men in **1981**, in particular for the development of methods to study single neurons in the primary visual cortex. This connection between Anatomy and Physiology will be an important theme in the fourth article.

In this part, we have seen how electrophysiology went from the analysis of large axons to how different cortical regions worked. It is a field that has laid the foundation for modern neuroscience. Vernon Mountcastle is seen by many as the first modern neuroscientist and he is also the founder of cognitive neuroscience, a fascinating field concerned with understanding how different cognitive processes work using methods in both psychology and neuroscience. We will learn a lot more about it in the next article.

Acknowledgement: I would like to thank Stefi Komala for her help with designing and Prof Andreas Nieder for help doing research for the article



References

Have scientists actually brought

WRITTEN BY AMINA IGENBEK
EDITED BY ANASTASIIA TARASENKO
DESIGNED BY DORIS YU

This May, researchers at the **Moran Eye Institute** and the **University of Utah** published a paper in Nature entitled "**Revival of Light Signalling in the Postmortem Mouse and Human Retina**". This paper sent shockwaves not just throughout the scientific community but also beyond it, and articles with catchy headlines such as "**Death Could be Reversible, as Scientists Bring Dead Eyes Back to Life**" and "**One step closer to immortality: Dead eyes brought back to life in Frankenstein-like study**" permeated major news websites. While it may be exciting to imagine these scientists as Dr Frankenstein-like figures, electrifying donor eyes suspended in questionable liquids and bringing whole organs back from the dead, this is, of course, not the case. **So, what exactly do these findings mean, and how will they affect research going forward?**

To understand this, we must first address what the authors were attempting to achieve with these experiments. According to this paper, "**Death is defined as the cessation of circulatory, respiratory, or brain activity**". It is well-established that, after loss of circulation, the central nervous system (CNS) loses viability within minutes, much quicker than other, peripheral, organs. So, while some organs can be donated after cardiac death by utilising methods of prolonging viability, the components of the CNS cannot. We still don't know why exactly the CNS loses viability so fast. In this paper, researchers used **mouse and human retinas**, which are the part of the eye that **receives signals in the form of light** and **translates them into electrical signals to the brain**, to study the kinetics of cell death in the CNS and test the hypothesis that it would be possible to **revive light signalling in photoreceptors** (which receive the light signal) and **ON bipolar cells** (one of the cell types that receives signals from the photoreceptors).

SHALLOW DIVE

human eyes back from the dead?

This hypothesis was informed by a **2019 study** where researchers were able to **induce spikes of electrical activity in postmortem pig brains** up to 4 hours after the animal had died. While this study showed that it was possible to activate isolated brain cells, they were unable to induce whole-brain activity. These findings suggested that **neuronal death may not be as irreversible as once thought**. Armed with this knowledge, the researchers set the ambitious goal of restoring not just the activity of cells but also the communication between these cells, much as they would in a live animal.

And they were able to achieve just that in the mouse retina. They found that, by putting the retina in an environment that closely mimics the conditions inside the body, they could **partially restore the functioning of the retinal cells** up to 3 hours after the animal had died.

They then set out to figure out what exactly was driving the rapid loss of function caused by the cessation of blood flow and found that the main culprits were **hypoxia** (a lack of oxygen) and **acidosis**, which happens because of a build-up of acids. But, as was pointed out in the paper, this was the easier part.

The human retina is a bit more complex than that of a rodent, and it has a **higher energy demand**, meaning it is safe to assume it would be more challenging to restore its function once the organ has died. And this is precisely what happened: while there was some activity in lone cells up to 5 hours after the retina was procured, the problem of making these cells talk to each other persisted. The previous results indicated that the main reason for this irreversible loss of activity was hypoxia, which occurs very early after death.

So, to solve this problem, working very closely with organ donation organisations, the researchers had to make sure they recovered the eyes very early after donor death (less than 45 minutes) and immediately put them in an environment that had high oxygen concentrations. By tweaking their method, they found they could almost **fully restore the function not just of separate cells but of the neural network as well**, showing quite miraculous results.

In what will surely become a landmark paper, **Abbas and colleagues have succeeded in restoring the function of the human retina up to several hours after the donor's death**. This is guaranteed to have far-reaching implications for multiple fields of study. First of all, there are still many unknowns surrounding the way our vision functions and how exactly diseases like age-related macular degeneration (AMD) come about. We've been using retinas from macaques to study this, but they aren't perfect models for the functional OR the disease state. Having **functional retinal material to study** from actual humans is sure to be a game-changer in addressing our unanswered questions in the near future.

These findings also lay the groundwork for **revolutionising eye transplants and grafting** (taking just the donor retina/cells and transplanting them into a diseased eye), potentially curing some forms of vision loss and blindness and prolonging the time a tissue can be viable after the death of the donor. While this may be far off, it is an exciting prospect.

Finally, this study **calls into question our very understanding of the irreversible nature of brain death**. If it was possible to revive eye cells, could other types of brain cells be next? This is surely a question scientists will be scrambling to answer moving forward. Of course, this is in no way the key to immortality or reversing death itself that some headlines will have you believe. But it doesn't have to be - what these researchers have managed to do is exciting and revolutionary, and it has already furthered our understanding of neural death and vision research in a way we couldn't have imagined even a few years ago.

References

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SHALLOW DIVE

WRITTEN BY ANNIKA ROBIOLIO EDITED BY KIRA LINKE DESIGNED BY YUSRA-AINA CHOUDHURY

WHEN WE FALL ASLEEP

WHERE DO WE GO?

What happens to our mind when we close our eyes, abandoning our relation with the outside world through sleep? In most cases, the only way to understand where our thoughts are directed is through the recollection of dreams. Even though we may not remember them, some wake up in a better or worse mood depending on the events of their dream. Some may have dreams so vivid, that it is difficult to distinguish them, creating a mix of the fictional story and the real world memories. But **what are dreams?**

A recurrent theme in Neuroscience is the attribution of **consciousness** to different mental states like imagination, wakeful reasoning and dreaming. Dreaming can be considered an **altered state of consciousness** in which fictional events occur and create an illusory story in our minds. Philosophy regards our involvement in the actual sensorial experience in the dream, whilst a scientific purpose regards the 'form' of dreams, i.e. what is the brain activation pattern, as well as the use of dreams as a **hallmark for mental health**.

Imagine having a dream that you are climbing a mountain. Could it be said that we have experienced climbing it? Or gained some form of knowledge? We cannot simplify the memory to mere imagination as we do not feel like we are the directors of the action around us, just like in real world situations. To understand what processes occur in our brain when we sleep we can use **fMRI** methods to visualise the activation of certain brain areas. Studies have shown high brain activity in areas like the **amygdala,**

thalamus and brainstem linked with visual hallucinations and emotion intensification. The neuropsychology of dreams has been debated extensively between the theorists J**. A. Hobson and M. Solms,** especially with regards to rapid-eye-movement (REM) sleep, a phase linked particularly with dreams. Hobson theorised that dreams originate in the **forebrain** together with the REM phasic firings, in the form of **ponto-geniculo-occipital** spikes, from which the brain tries to make sense of the casual eye movements by creating a story narrative that follows these movements. This suggests that dreams arise from the **decoding of the chaotic REM** movements in visual pathway areas coupled with areas linked to emotions like the hypothalamus. This leads to the formation of the dream narrative. From this view, dreams do not have much conscious scenery-creation involvement within them, but rely purely on the interpretation of rapid-eye movements.

In contrast, **Solms** studied the activation patterns of the prefrontal cortex areas, linked with higher order thinking, in brain-injured patients. He tackled **Hobson's theory** by showing that these areas of activation in sleep reflect those of higher thinking experienced when we are awake. From this view, dreams are not only REM signal interpretations but are actually **spontaneously arising firings** in the prefrontal cortex. The proof of this was finding that dreams happen also in REM sleep absence, so when there is **no chaotic eye movement** to interpret, even if in a lower frequency.

The prefrontal activation suggests that while dreaming we have the will to perform an action, but this does not happen due to a motor block that separates our behaviours in the dream from the ones in the real world. Therefore, an **impaired motor block** could be the reason why some people sleepwalk or talk. It has also been shown that the environment in which one dreams influences the content of the dream. So, when we are studying consciousness during sleep, it is important not to forget that in a limited way our body is still affected by the real world environment around us, even if we do not interact with it directly due to the inability we have to perform voluntary movements while sleeping.

Brain activation studies have also been performed on people who reportedly experience **lucid dreaming** through a combination of **fMRI/EEG**. Lucid dreaming involves the capacity of the subject to direct their own actions during the dream and be in control of the scenery. These scans show greater activation of areas linked with higher thinking in the prefrontal cortex than normal dreaming. The most notable difference between normal REM sleep and lucid dreaming was seen in the precuneus region, a brain area that is related with self-referential processes. In fact, dreams are usually set in external sceneries while lucid dreams often reflect our own state of mind. It is therefore clear that what makes the dream experience differ from other forms of experience is the awareness one has of being in a dream state.

We have memories of some dreams but perhaps dreams can happen even if we do not recall them: the explanation for this could stem from cognitive access.

Cognitive access is the ability of our mind to engage in cognitive functions while being aware of this engagement. During dreaming, our mind could engage in higher thinking cognitive functions but with a reduced awareness of this process. This phenomenon can be supported by brain scan fMRI. These scans showed a reduced activation of the **dorso-lateral-prefrontal cortex**, in comparison with awakened subjects. Therefore this area has been putatively linked with the ability of our mind to be aware of the use of cognitive functions, explaining the difference between dreaming and other conscious states. A summary of the most relevant brain areas mentioned till this point can be seen in figure 1.

Dreaming is a method for our brain to **rehearse real world situational behaviour**, reorganise thoughts, or even to rearrange memories. In fact, memory consolidation has been linked to sleep and dreaming. The way in which our memories are formed is through neural circuits strengthening, through repetitive stimuli presentation to which our brain consolidates connections. This idea was first introduced by Lomo, in 1966, through the observation that repetitive single stimuli were able to produce an increasing number of EPSP in a given synapse. This phenomenon was called long term potentiation. Although this experiment was initially thought for the study of memory in the hippocampus, it has now been shown that most areas of the brain use this mechanism. Therefore, we can say that these connections are the basis that underlies the way we reason, we behave and we think in relation to the external world.

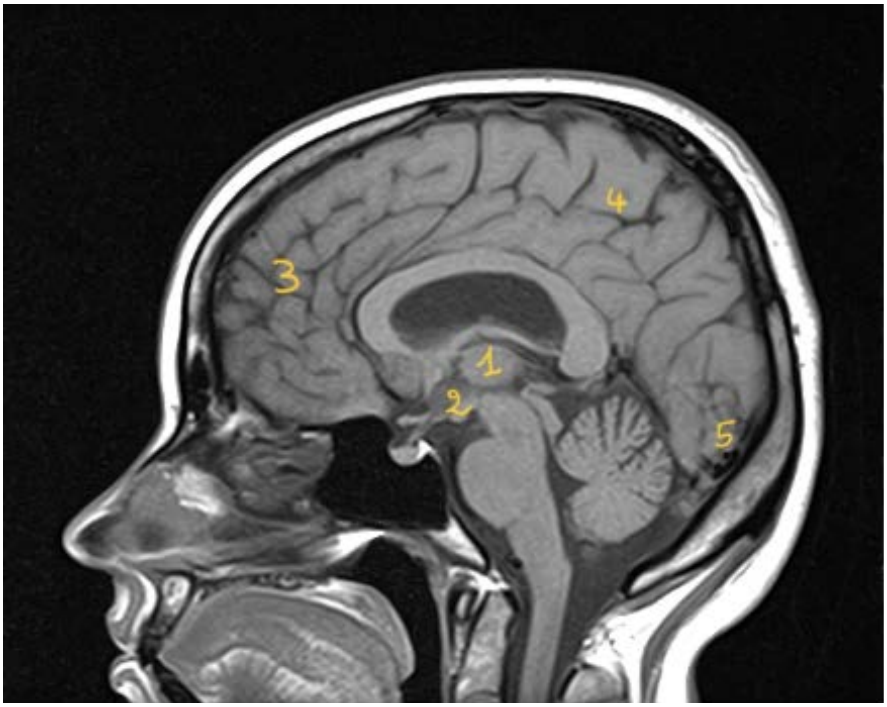


Fig.1 shows a PET brain scan from Haouimi, A. (2019) and highlight main brain areas involved in dreaming, with adaptation from Nir, Y., & Tononi, G. (2010)

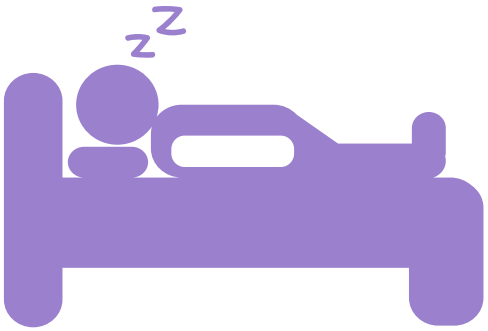
1- thalamus
2-limbic structures including amygdala and hippocampus
3- medial prefrontal cortex
4- precuneus and posterior cingulate
5- visual cortex

New connections are always formed and shaped by the various experiences we encounter in our life. If dreaming is connected to synaptic connection straightening, it is evident that even though we might never understand completely what happens in our minds when we sleep, we cannot deny the experience-like impact that some dreams leave on us when we wake up from them.

If we consider sleep disturbances like **nightmare disorder**, we can see how dream experiences have to resemble external world experiences in our consciousness. Here, the subject tends to avoid sleep in order to not face recurrent nightmares. If we consider that sleep deprivation leads to dramatic health implications, we can hypothesise that nightmares have to at least resemble real-life experiences, if the subject avoids such a fundamental need like sleep. However, one might question whether it is the dream experience that is avoided or the real-life-like memories that arise from the dream. In turn, poor sleep patterns are linked with mental disease. Therefore, dreams can be an indication that a mental health issue might be present and they can even influence mental health problems. For example, studies have shown that participants who reported scarier dream narratives had a **correlation with lower arousal levels in real life** in stressful situations. So, scary dreams lead to healthier behavioural responses. Having said that, a study by **Titus et al,** showed a correlation between scary dreams in schizophrenic patients and an increase in dangerous negative symptoms.

Another study by **Schredl et al**, showed that vivid dreams in children were correlated with a higher generalised anxiety; with sources of aggression within the dream indicating forms of fear in real life. Through these examples it appears undeniable that there is an intrinsic link between dreams and our mental health, as elements in dreams seem to be a reflection of our awakened mental states.

Our current understanding of the neuroscience of dreams gives us an indication of potential areas involved. That, however, is not able to fully answer questions regarding the 'form' of dreams. What they are, why we dream, and how much our thoughts and emotions are involved in them. But other than the fascination towards this mysterious conscious paradigm, continued study is important as they give space to reflect on mental health. Dreams could be seen as a free conscious stream, without the blocks imposed by our awareness of emotions and intentions. This could help us understand ourselves better, and help us find out how our mental states are when we cannot control them.



References

TREADING WATER

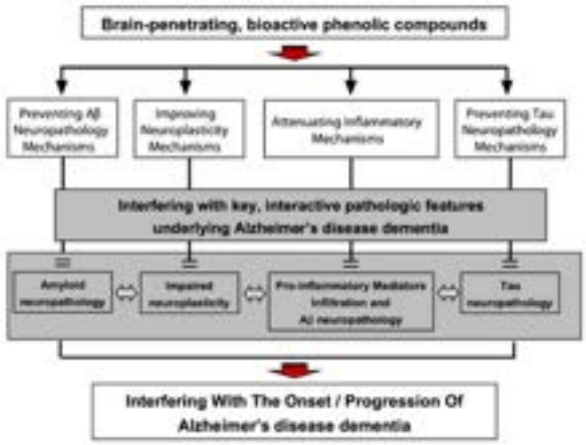
Polyphenols and their link to Alzheimer's Disease - A Novel Therapeutic Area?

WRITTEN BY ZETA IOANNOU
EDITED BY NICOLA ALLEN
DESIGNED BY SICHUN YAO

Having the privilege of being Team Leader this year for **KCL iGEM** has allowed me to research several topics in depth relating to **synthetic biology** and beyond. One important topic that I would like to shine light upon is that of **Alzheimer's Disease (AD)** and novel therapeutic areas relating to it; specifically the use of **polyphenols** in AD treatment.

In order to understand the reason why AD therapeutics need further development, one ought to understand the current **limitations** of existing treatments. It is worth noting that for the palliative treatment of AD, **only four** drugs are approved by the FDA. These are three **acetylcholinesterase (AChE) inhibitors** and one **N-methyl-D-aspartate (NMDA) receptor antagonist**; these help alleviate symptoms but do **not** prevent the progression of cognitive deterioration. The fact that AD is only treated **symptomatically**, has given rise to different areas of research which focus on **slowing down** the progression of AD at its **earliest** stages.

AD is characterised by the **build-up** of proteins in the brain areas, starting off usually at the **hippocampus**. They consist of two main types: **beta-amyloid (Aβ) plaques** outside neuronal cells and **Tau tangles** within these cells. They cause **neuroinflammation** and **cell death**, ultimately leading to **cognitive decline** that can often be experienced as **memory loss** as one of the key symptoms. **Polyphenols** are a category of compounds naturally found in plant foods, such as fruits, vegetables, herbs, spices, tea, dark chocolate, and wine. Polyphenols act as **free radical scavengers**, and thereby provide protection from chronic diseases, such as AD, in which the free radicals play major roles in its pathogenesis.

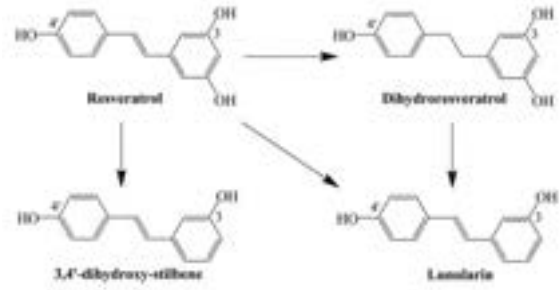


Schematic showing the key role that polyphenolic compounds play in targeting the underlying pathophysiology of Alzheimer's Disease. As can be seen, polyphenols target more than one aspect of the pathogenesis (Adapted from Rocha-González et al., 2008).

To emphasise this, according to Ho et al. (2009), **resveratrol**, a polyphenol that has been shown to improve cognitive ability in-vitro and in-vivo, may help attenuate AD by modulating **Aβ neuropathology**. This is done through the inhibition of both Aβ generation and abnormal Aβ oligomerisation, and through the promotion of Aβ clearance by modulating **tau neuropathology**, mainly via the inhibition of abnormal tau phosphorylation and aggregation. All of these Aβ and tau mechanisms are key therapeutic targets for AD which, if interrupted, would not only alleviate symptoms of AD, but also directly target its pathophysiology to slow down its progression (Pasinetti et al., 2015).

In addition, recent fractionation studies have also revealed that a **grape seed polyphenolic extract (GSPE)** is capable of significantly attenuating AD-type phenotypes in transgenic mice, primarily due to its capacity to increase the bioavailability of **flavan-3-ol molecules** (e.g. catechin, epicatechin, etc.) in the brain (Pasinetti et al., 2015). Other studies revealed that resveratrol may promote intracellular **Aβ clearance**, in part by activating autophagy and AMPK signalling in-vivo (Vingtdeux et al., 2011). Overall, outcomes from these studies support the notion that autophagy and inflammation work in concert with respect to the **anti-amyloidogenic** effect of resveratrol.

Even though resveratrol has been shown in-vitro to have beneficial effects against AD (Rocha-González et al., 2008), it has significant limitations when considered clinically such as its **low bioavailability** and its decreased ability to readily cross the **blood-brain barrier**. Due to this, attention has now shifted towards metabolites and derivatives of resveratrol such as **pterostilbene**, which has an overall bioavailability of approximately **80%** compared to resveratrol's 20%. Moreover, in-vitro and **in-vivo** pharmacological activities of pterostilbene are usually found to be stronger than that of resveratrol (Wang and Sang, 2018).



Pathways showing the metabolic link between resveratrol and pterostilbene (3,4'-dihydroxy-stilbene). Adapted from Wang and Sang (2018).

All in all, there seems to be an increasing link being made in the literature between the pathophysiology of AD and the administration of polyphenols. Nevertheless, due to limited clinical data investigating this relationship, accurate conclusions **cannot** yet be determined. This up and emerging field should be of extreme importance to future researchers investigating AD treatments, as it poses a way to revolutionise AD healthcare as we know it, targeting AD at its **core** and **not** simply just its **symptoms**.

References

TREADING WATER

Photo by National Cancer Institute on Unsplash

Are combination anti-HIV antibodies effective in suppressing HIV for extended periods?

WRITTEN BY PRACHI PURANIK | EDITED BY ANDREA MAZGALEVA | DESIGNED BY DORIS YU

Human immunodeficiency virus (**HIV**) is said to have originated in chimpanzees from parts of West Africa in the 1930s. However, the world only came to know of the virus in the 1980s when doctors began to report rare types of illnesses. In 1987 the U.S Food & Drug Administration (US-FDA) approved the first antiretroviral drug, **zidovudine**. Over the years several drugs have been discovered which are used in combination with other anti-HIV drugs to treat HIV through **antiretroviral therapy (ART)**.

ANTI-RETROVIRAL THERAPY (ART)

To understand how ART works it is important to understand HIV. When the virus **infects** a particular cell, it **takes full control** of that cell and uses it to make more copies of itself. **Antiretroviral drugs** such as zidovudine are used in combination with other anti-HIV drugs, usually from different classes to **stop the virus replicating** in the body.

There are **6 drug classes**: Nucleoside Reverse Transcriptase Inhibitors (**NRTIs**), Nonnucleoside Reverse Transcriptase Inhibitors (**NNRTIs**), Protease Inhibitors (**PIs**), Fusion Inhibitors (**FIs**), **CCR5 antagonists** and **Integrase Inhibitors**. All these classes have a different mechanism of action to inhibit replication, making it more effective in treatment.

Although the antiretroviral therapy reduces the effect of HIV, **it is not a cure**. There are several barriers to universal uptake of ART such as having a medical regime everyday. These pills can also have long-term side effects and pose the possibility of developing a drug-resistant virus. It is also possible for viral rebound to occur within weeks of discontinuing ART which makes the patient in need for lifelong adherence.

BROADLY NEUTRALISING ANTIBODIES IN CLINICAL DEVELOPMENT

In a search for ART-free strategies multiple broadly neutralising antibodies (**bNAbs**) that **target vulnerable sites** on the **HIV envelope** are being assessed. Clinical trials have shown that viral rebound can be delayed significantly by bNAbs even after taking the patients off ART. This is more likely if the patients had started ART in early stages of their infection.

Previously single bNAbs showed little success in suppressing the virus as bNAb-resistant virus either already existed in the patient or was developed. To avoid this setback, researchers in the National Institute of Allergy and Infectious Diseases (NIAID) developed a **combination bNAbs** which targeted different parts of the virus envelope. This was successful in suppressing the virus for a longer period without ART.

But the researchers faced a major challenge as bNAbs proved to be **ineffective** in suppressing HIV if the patients had a HIV strain which was **resistant** to one or both of the antibodies.

NEXT GENERATION BNABS

Going forward infrequent administration of antibodies along with long lasting ART antibodies is definitely possible on finding more potent and durable drugs and antibodies. These strategies will need to be further assessed in phase II and III of the clinical trials. The ultimate goal would be to **identify antibody combinations** which can also cover circulating variants so that screening for existing resistance to antibodies in HIV can be eliminated.

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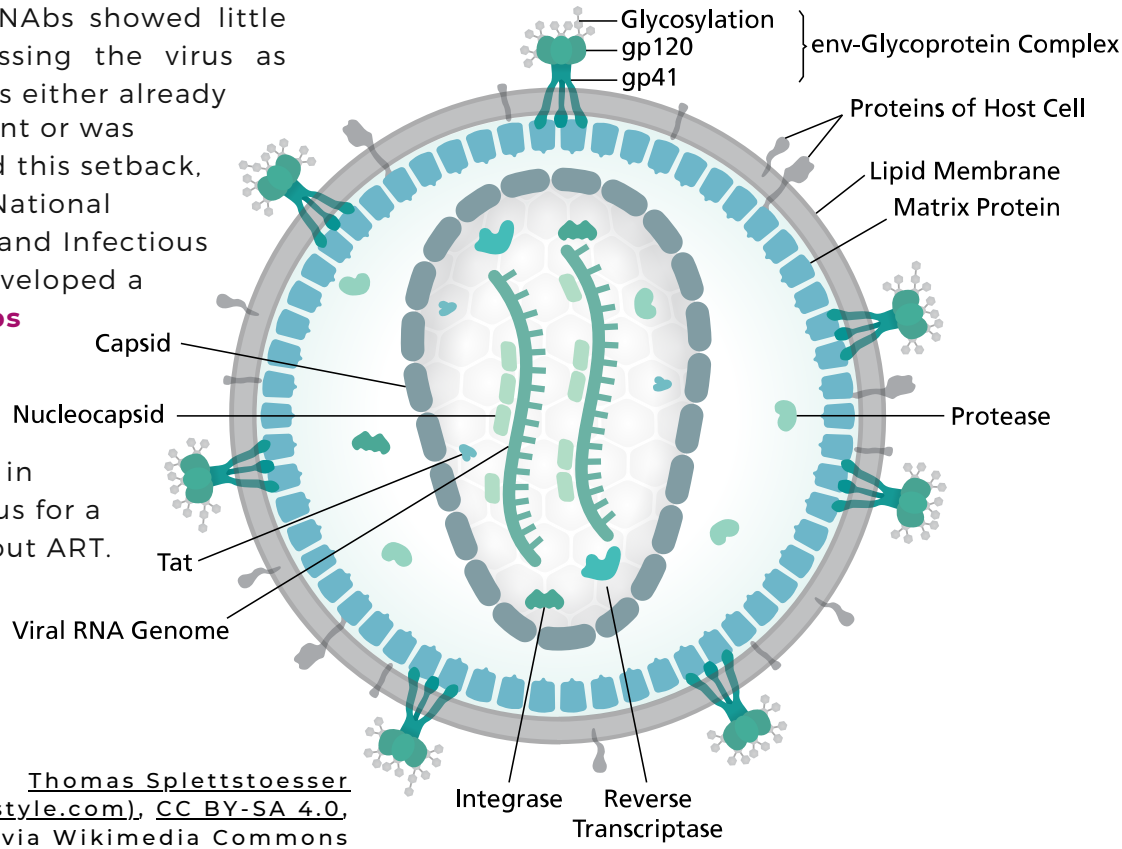

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SHALLOW DIVE

CHILDS FAMILY SEEKS $75,000 IN DAMAGES FOLLOWING AIRPODS-INDUCED HEARING LOSS

WRITTEN BY ANDRES JARAMILLO MOYANO
EDITED BY AMINA IGENBEK
DESIGNED BY YUSRA-AINA CHOUDHURY

Ever since Apple introduced their famous AirPods in 2016, several companies have come out with their own iterations. It's no surprise that the product has been a massive success, with Apple taking a 26% share of wireless headphone sales in 2016 and 31% in 2020, following **100 million sales.**

However, Apple is currently facing a **$75,000 lawsuit** where a child's family alleges that their **12-year-old** son had their **eardrums shattered** while wearing their **Airpods Pro**. The couple from San Antonio, Texas, claims their son was left partially deaf following an incident where the boy was watching a movie on Netflix at a low volume when suddenly an 'Amber alert' was issued. The family claim this caused significant damage to the boy's hearing after tearing his eardrum and damaging his **cochlea** (the hollow, spiral-shaped bone in the inner ear which is vital in transducing sound waves into electrical impulses that the brain interprets as individual frequencies of sound). In this lawsuit, the family claims that the boy has suffered from **vertigo, dizziness, tinnitus and nausea** since the incident.

This article aims to investigate the claims based on the scientific understanding of how our ears work, and look at both sides of the argument in this lawsuit.

See, our ears are fine-tuned organs which are very sensitive. It all depends on how often you are exposed to certain noise levels. The **decibel scale**, for example, ranges from **0dB (barely audible) to 130dB (pain threshold)**. To put this into perspective, your eardrums are capable of withstanding up to 85dB (decibels) for around 8 hours a day, but noise over 85dB (or 85dB exceeding 8 hours a day) is capable of damaging your hearing. **The World Health Organisation (WHO)** recommends 70dB as a safe threshold for human hearing, equivalent to the sound of traffic, whereas 85dB would be the same as the volume in a noisy restaurant.

On average, AirPod wearers have a listening level of 94dB, but is it plausible that an emergency alert on an iPhone can cause a ruptured eardrum? Well, let's first address what 'Amber Alerts' are. 'Amber Alerts' are a type of warning message automatically displayed on smartphones alerting residents of a child abduction in the area. They usually include details surrounding the victim, the suspect and any vehicles used.

In this particular lawsuit, the plaintiff claims that Apple's audio accessories like AirPods don't automatically lower the volume for alerts. According to **appleinsider.com**, user reports corroborate that 'Amber Alerts' are not equalised to the volume of the content that someone may be listening to and that these alerts do play loudly on AirPods.

On the other hand, one would have to look at the volume output of AirPods and see whether they match the decibel level required to rupture an eardrum. According to the **NHS**, a perforated eardrum can occur due to a sudden, very loud noise like an explosion or a gunshot **(usually 165dB or more)**. While painful, it should only **affect hearing temporarily** as it can heal itself within a few weeks. However, bacteria and other pathogens may get into the middle and inner ear, triggering an infection which can cause permanent hearing damage. Since both the **iPhone** and **AirPods** have a **maximum** volume output of **100-110dB**, the argument that the AirPods would have been able to cause sudden considerable damage such as rupturing the wearer's eardrum does not hold up in theory. To corroborate this, users can inspect their headphone safety in the **'Sounds & Haptics'** section in the settings app, where it shows that headphone audio peaks at 100dB. Even at this volume level, it would not rupture the eardrum.

To round off, there is limited information on the lawsuit as it's still ongoing. Key details such as what volume the user had their AirPod Pro earbuds set to prior to the incident as well as what decibel level the 'Amber Alerts' peak at on AirPod Pro's have yet to be determined. Until the verdict is out, we can only speculate on the plausibility of this incident.

References

How is GSK innovating the field of immuno-oncology?

DEEP DIVE

WRITTEN BY LEON ZHANG
EDITED BY ASTRITI LAKSHMI ADITYA
DESIGNED BY DORIS YU

Oncology is the scientific field that studies the causes and treatments of **cancer**, a disease caused by the uncontrollable division of abnormal cells, forming tumours within the body. Throughout the eighteenth and nineteenth centuries and coinciding with the development of anaesthesia, surgical techniques were developed to remove benign tumours that were comprised of non-invading cells enclosed within connective tissue ("understanding What Cancer Is: Ancient Times to Present," 2019). Malignant tumours, whose cells migrated to distal sites to form secondary tumours (metastases), and surgically inaccessible tumours within the brain, however, remained inoperable ("understanding What Cancer Is: Ancient Times to Present," 2019). The last few decades have revolutionised our understanding of cancer and its treatments as highlighted poignantly through **Hanahan and Weinburg's** seminal paper on the **hallmarks of cancer** and the development of **radiotherapy**, **chemotherapy** and **immunotherapy** which have replaced or are used in conjunction with surgery. Currently, the next generation of immunotherapies is being developed by **GlaxoSmithKline** (**GSK**) which has acquired the rights to antibodies targeting the **CD266 axis** through strategic licensing deals.

WHAT IS IMMUNOSURVEILLANCE AND WHY IS THERE A NEED FOR IMMUNO-ONCOLOGY?

Genetic factors such as inherited mutations and **environmental factors** including exposure to harmful radiation, mutagenic agents and viruses are known to contribute to cancer. Mutated, viral and abnormally expressed proteins are presented on **MHC1** molecules at the cell surface membrane where they activate **natural killer** (NK) cells and **dendritic** cells which contributes to the destruction of some cancerous cells and the secretion of cytokines that activates **antigen-specific T- and B-cells** (Finn, 2012). These cells contribute to the **adaptive immune response** and result in cytotoxic T-cell mediated and antibody-mediated depletion of tumour cells, respectively in a process known as **immunosurveillance** (Finn, 2012). Cancer cells, however, express **immunosuppressive** molecules leading to inhibition of the anti-tumour-specific immune response and failure to efficiently clear cancer cells and the formation of a dysfunctional/exhausted immune cell population (Yeo, Ko, Lee, Park, & Jin, 2021). This has led to the development of **immuno-oncology**, a form of immunotherapy that involves using small molecules or biologics to strengthen a cancer patient's immune system to recognise tumours or provide missing immune effector functions (Finn, 2012). Currently, **monoclonal antibodies** that inhibit checkpoint proteins, such as PD-1, PD-L1 and CTLA-4, are used. These checkpoint proteins regulate the immune response (Finn, 2012).

THE CD226 AXIS

Currently, **less than 30% of cancer patients benefit from checkpoint inhibitor drugs**, thus emphasising the need for alternative targets such as the CD226 axis ("Advancing the next generation of immuno-oncology," 2021). CD226 is a **transmembrane protein** present on NK and T-cells that binds to CD155 (PVR) and CD112 (nectin-2) to stimulate an **anti-tumour immune response** (Yeo, Ko, Lee, Park, & Jin, 2021). **PVR** and **nectin-2** are **upregulated** in cancer cells and also act as **ligands** for **immunosuppressive checkpoint proteins** such as CD97, PVRIG and TIGIT which, together, form the **CD226 axis** which is necessary to immune evasion (Yeo, Ko, Lee, Park, & Jin, 2021). Exhausted NK and activated T cells upregulate TIGIT expression to compete with CD226 for PVR and nectin-2 binding, thus **antagonising** CD226-mediated anti-tumour response (Chauvin & Zarour, 2020). PVR and nectin-2 ligand binding can also result in the production of immunosuppressive dendritic cells and regulatory T-cells that secrete anti-inflammatory interleukin-10 to reduce NK cell-mediated cytotoxicity and T-cell effector functions (Chauvin & Zarour, 2020). Similarly, NK cells and T-cells also express CD96 which binds to PVR to **antagonise** CD226 signalling, inhibit interferon-gamma production and metastases clearance (Dougall, Kurulus, Smyth, & Anderson, 2017). PVRIG (CD112R) is also expressed which competes with CD226 for nectin-2 binding, resulting in dampened effector cell activation (Panduro et al., 2020).

NEXT-GENERATION IMMUNOTHERAPIES TARGETING THE CD226 AXIS

GSK is uniquely positioned as a leader in the field of immuno-oncology as they are the only pharmaceutical company to **acquire the rights to antibodies targeting** CD96, PVRIG and TIGIT which comprises **the CD226 axis** ("Advancing the next generation of immuno-oncology," 2021). These antibodies can bind to their target receptors to block PVR and nectin-2 binding, thus promoting CD226-mediated NK and T-cell activation which is necessary for the anti-tumour response. They also recruit macrophages and cytotoxic cells to CD96+/PVRIG+/TIGIT+ cells through their Fc domain, resulting in antibody-dependent cell-mediated phagocytosis and cytotoxicity.

ANTI-CD96 ANTIBODIES (GSK'608)

In 2018, GSK purchased **$300 million** in shares of **23andMe** as part of their four-year partnership deal to co-develop drugs targeting novel molecules identified using the 23andMe's vast genetic database ("GSK and 23andMe sign agreement to leverage genetic insights for the development of novel medicines." 2018). This collaboration has been successful and led to the development of **GSK'608**, an anti-CD96 human IgG1 monoclonal antibody which is now in phase 1 clinical trials. The partnership was extended by an additional year in early 2022 with a different set of terms – in exchange for the sole responsibility of developing and commercialising GSK'608, 23andMe would receive an upfront $50 million payment in addition to future royalties on GSK'608 sales ("23andMe Announces Extension of GSK Collaboration...," 2022).

ANTI-PVRIG ANTIBODIES (SRF813)

SRF813 is an anti-PVRIG human IgG1 monoclonal antibody that is owned by Surface Oncology and is currently in preclinical studies ("Surface Oncology Announces Exclusive License Agreement with GSK for Novel Immunotherapy Program," 2020). It was exclusively licensed to GSK in 2020, in exchange for $85 million in upfront payments, a possible $730 million in milestone payments, and royalties on global sales ("Surface Oncology Announces Exclusive License Agreement with GSK for Novel Immunotherapy Program," 2020).

ANTI-TIGIT ANTIBODIES (EOS-448)

Similarly, in 2021, GSK also announced the co-development and co-commercialisation of an anti-TIGIT human IgG1 monoclonal antibody (**EOS-448**) with iTeos Therapeutics.

In addition to a $625 million upfront payment, iTeos would also receive up to $1.45 billion in milestone payments and share profits in the US and receive royalty payments outside of the US where GSK will hold exclusive commercial licensing rights ("GSK and iTeos Therapeutics announce development and commercialisation collaboration for EOS-448, an anti-TIGIT monoclonal antibody, enabling novel next-generation immuno-oncology combinations," 2021). Preclinical studies and phase 1 clinical trials of EOS-448 reveal it preferentially binds to and depletes TIGIT+ regulatory T cell populations that contribute to the immunosuppressive tumour microenvironment (Preillon et al., 2021).

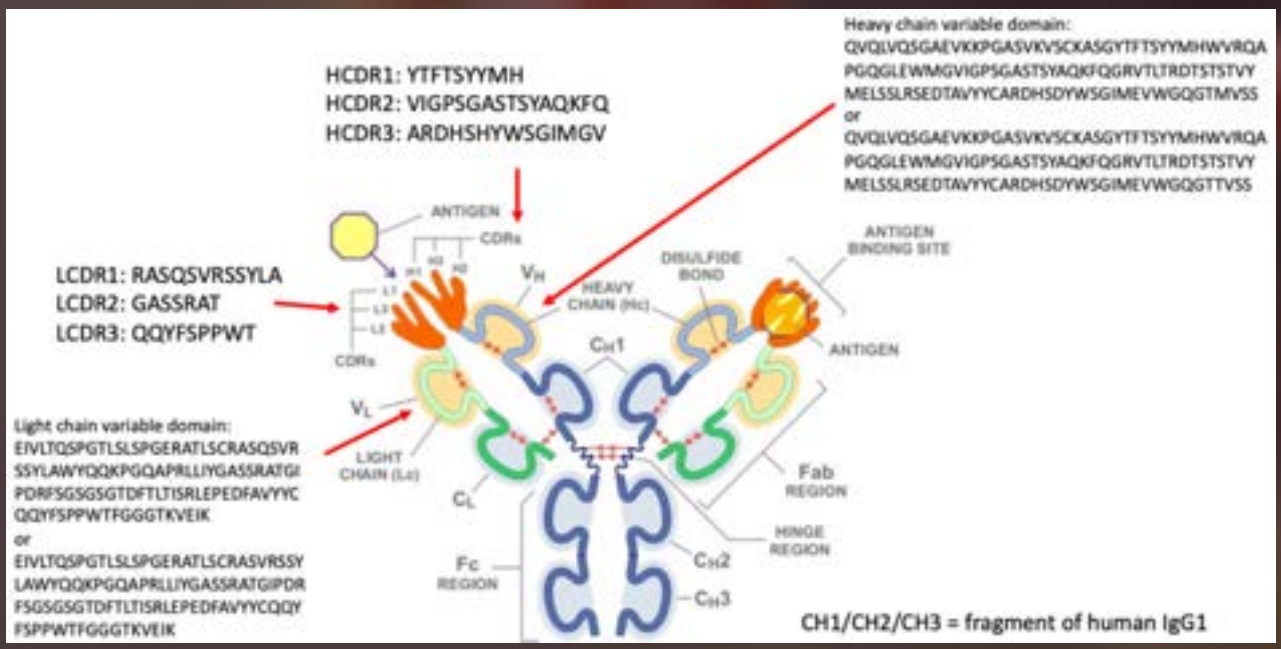

Figure 1. Summary of claims from the EOS-448 patent

TO WHAT EXTENT HAS GSK MONOPOLISED THE CD226 AXIS?

GSK is now the only pharmaceutical company to hold exclusive licensing rights to patented CD96, PVRIG and TIGIT antibodies (US20200199227A1, WO2020018879 and US20190100591, respectively). **Patents** are legal titles that grant holders the right to prevent others from making, using, selling or importing their invention without consent for up to twenty years in a geographical territory, in exchange for public disclosure of their invention; by definition, patents are a means of establishing a monopoly. In practice, the set of claims in a patent is rarely broad enough to prevent competitors from developing alternative products with similar functions. The EOS-448 patent (US20190100591) prevents competitors from developing an antibody/antibody fragment with an amino acid sequence similarity of at least 95% to the claimed sequences (Anti-TIGIT Antibodies, 2018). However, there are currently **eight other patented anti-TIGIT antibodies** in clinical trials with different amino acid sequences, target epitopes and functions (Chauvin & Zarour, 2020). The degeneracy of the amino acid code means that different amino acid sequences can adopt the same tertiary structure and exhibit the same function, thereby allowing the development of alternative anti-TIGIT antibodies. Furthermore, the EOS-448 patent does not protect against the development of antibody alternatives such as DARPins, alphabodies, affibodies and affimers whose small molecular weight allows efficient penetration of solid mass tumours.

CONCLUDING REMARKS

Despite having exclusive rights to these three antibodies making GSK a leader in the field of immuno-oncology, **there is nothing stopping competitors from developing alternative products targeting the same targets**. The purpose of the patent system is to drive innovation by providing inventors with a financial incentive for full public disclosure of their inventions. A brief search of anti-TIGIT patents reveals the development of bi-, tri- and multi-specific antibodies and monobodies by competitors. The biggest concern of a company monopolizing a specific market is that they will control the costs of products, in this case, medical treatments. However, it appears they are not alone in their goal of targeting the CD226 axis and competition will inevitably drive down costs.

References

SHALLOW DIVE

World's First Pig Heart

What's I Xenotransp

WRITTEN AND DESIGNED BY DO

In what has since been dubbed a '**landmark**' in medicine, the surgery team at the **University of Maryland School of Medicine** (UMSOM), led by **Dr Bartley Griffith**, performed the world's first pig heart transplant on **David Bennett Sr.** in **January 2022**. The team was granted special permission by the US Food and Drug Administration to perform the procedure on Bennett, who had terminal heart disease and was deemed ineligible to be added onto the heart transplant waiting list. Bennett's heart transplant marks the **first time a human receiving a pig organ is given the chance to survive and recover**.

Xenotransplantation – the process of moving tissues between species – is being researched as a potential avenue to relieve the current organ donor shortages experienced around the world. Last year it was recorded that **474 people died** while waiting for an organ transplant in the UK, with **over 6,000 people** currently on the **waiting list**. Bennett's operation follows two other human studies with pig organs conducted by the University of Alabama and New York University in late 2021, where pig kidneys were attached to clinically brain-dead patients.

The heart used in the surgery came from a genetically modified pig raised and engineered by **Revivicor**, a regenerative medicine company based in Virginia. The pig contained **ten genetic modifications** (**Figure 1**), where four pig genes were inactivated and six human genes were added. One of the pig genes was deleted to prevent the heart from growing once implanted, while the other nine modifications aimed to prevent immune rejection and facilitate organ acceptance. It is uncertain how many of these modifications were essential for transplantation.

A few days after the operation in January, Bennett was able to sit up and **showed promising signs of recovery**. The surgery team regularly performed a range of innovative tests to monitor Bennett and his new heart's condition. One such test used a **DNA sequencer** to scan his blood for fragments of pig genes, where an increase would indicate that the heart cells were dying. Another novel test developed by **Karius** screened his blood for traces of over 1000 pathogens.

For the first month, Bennett seemed to be doing well and was able to

Transplant Patient Dies

Next for plantation?

RIS YU | EDITED BY ALEXI MERY

spend time with his family, passing a critical milestone for transplant patients. But after about **40 days**, his condition began to deteriorate, and he **died in March 2022**. The researchers are still investigating the exact cause of death and what happened to the pig heart. In their examination, they found that the heart contained **porcine cytomegalovirus**, a pig virus that has been linked to **organ damage** and **devastating effects** on transplant patients; however, it is not believed to be capable of infecting human cells. The degree to which the virus contributed to Bennett's death is still undetermined, but Griffith believes it **"maybe was the actor, or could be the actor, that set this whole thing off"**.

It is also unclear where the virus may have come from, as the pigs were supposedly raised in a 'pathogen-free' facility. Revivicor has yet to make a public statement about this finding.

The discovery of the pig virus is not necessarily a major setback. If it played a role in Bennett's death, it could mean that **a virus-free heart xenotransplantation would last much longer**. Bennett was also very ill, which would not have improved his chances of survival. A biopsy of the heart **did not reveal obvious signs of immune rejection**, a promising finding given that this was a major concern for the researchers. **Dr Muhammed Mohiuddin**, scientific director of UMSOM's Cardiac Xenotransplantation Program, believes the procedure provided **"invaluable insights… that the genetically modified pig heart can function well within the human body while the immune system is adequately suppressed"** and remains positive about future work in the field.

Griffith shares a similarly optimistic view, and has said that while it is expensive to conduct xenotransplantation research (as it is usually tested in nonhuman primates), he is hopeful that this study will generate more commercial interest for further research funds. Ultimately, he hopes to find a way to **"reduce the amount of immune suppression required for a patient"**. The research team is currently writing up their findings for publication. **"He lived 60 days before he died, and we're not yet sure why. That is under intense investigation. Something happened to his heart, but we're not sure what. And that'll come out in time, and as we understand it better, we're certainly going to share it."**

Will xenotransplantation become a reality in the future? Perhaps, but there are still many unknowns that need to be answered before it would be deemed safe enough to test in clinical trials, let alone as a widespread therapeutic option. Bennett's transplant was the first major step towards unlocking the potential of xenotransplantation, but continued research in the area still faces **significant cost and ethical barriers**. Two major issues surrounding the **moral status** of animals raised specifically to provide organs for human use and whether a patient waiting for an organ transplant can really give **informed consent** have already been extensively debated in the literature. Xenotransplantation is an expensive research avenue that may only realistically benefit a select few, so it is worth considering whether society's resources would be better spent on treatments and preventative measures that help the larger population.

As is usually the case in discussions about the ethics of medical research, there is no objective right or wrong answer. But if properly researched and developed, xenotransplantation could one day **alleviate the strain on organ transplant resources**. Bennett's son, in an obituary to Bennett released by the University of Maryland, shared similar sentiments, **"We hope this story can be the beginning of hope and not the end. We also hope that what was learned from his surgery will benefit future patients and hopefully one day, end the organ shortage that costs so many lives each year."**

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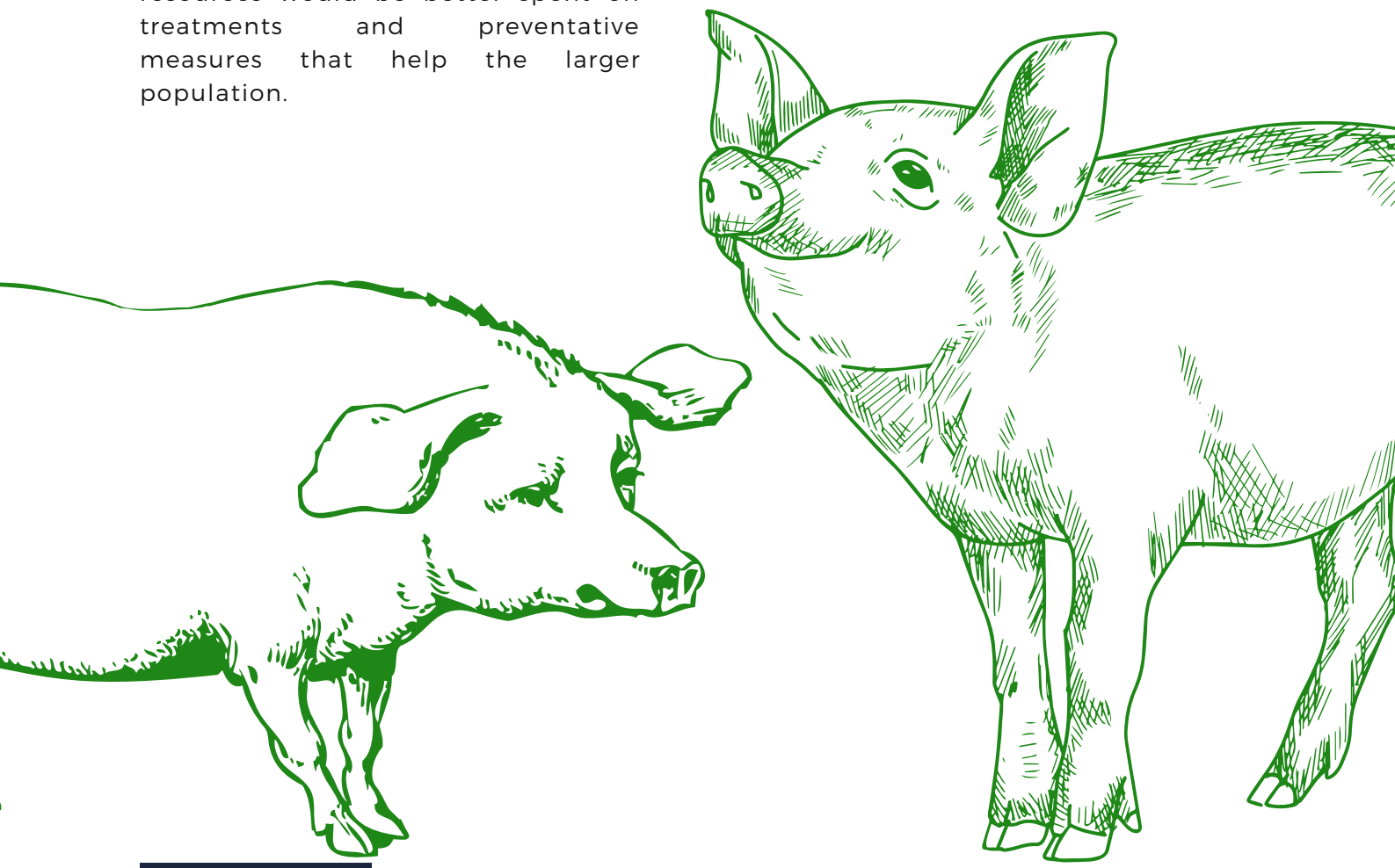


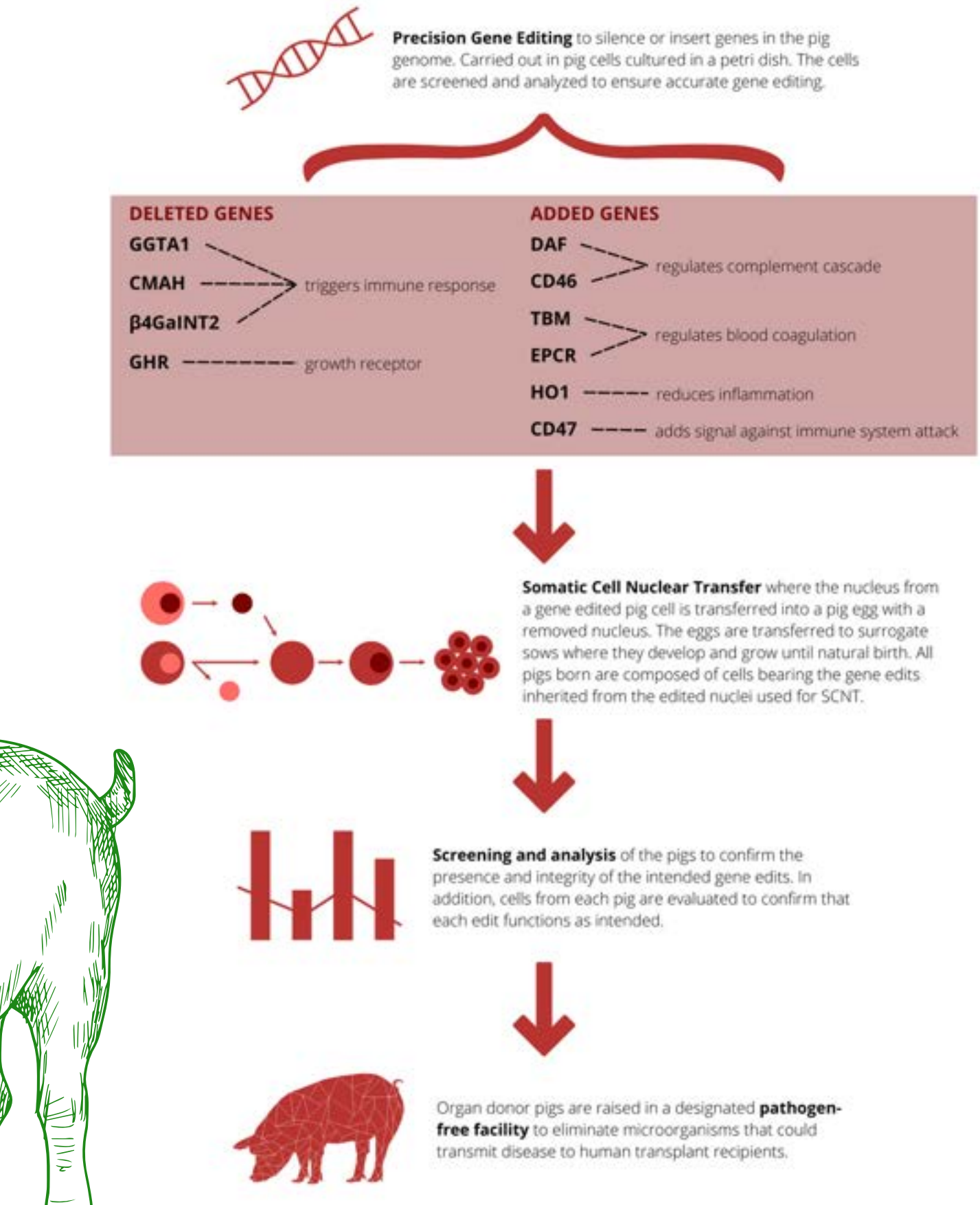


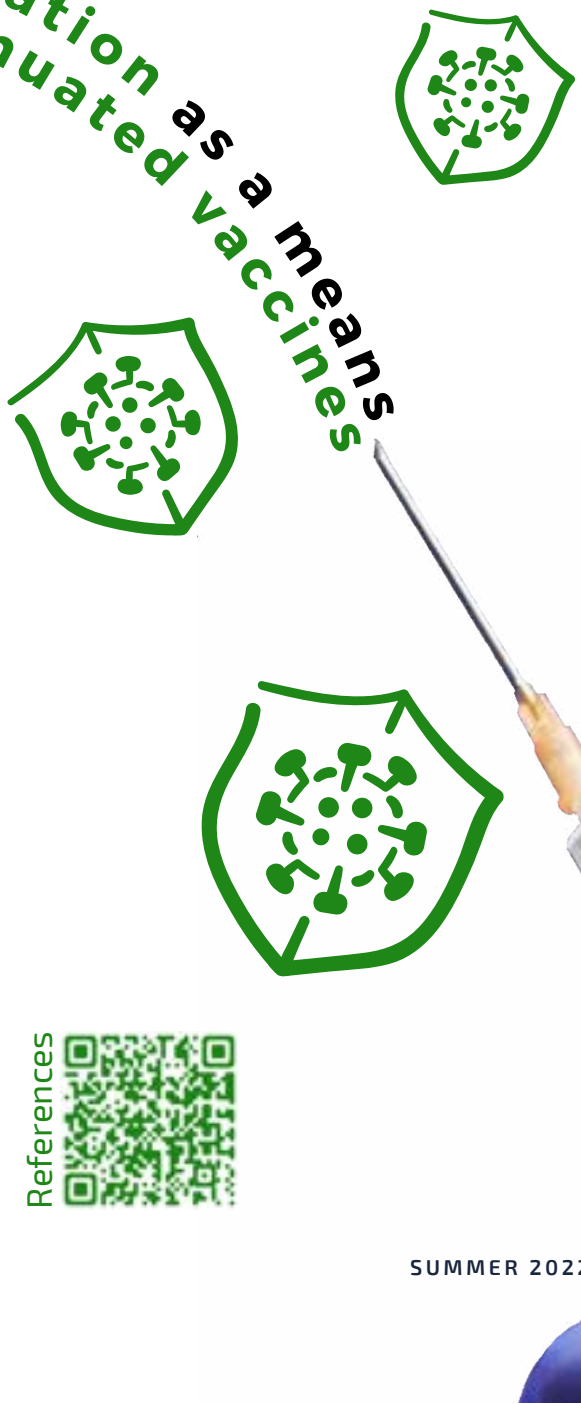
Figure 1. How the genetically modified pigs used were engineered for xenotransplantation. Adapted from Revivicor.

Codon deoptimization as a means of creating attenuated vaccines

Synthetic Biology more than any other discipline in the life sciences relies on thinking outside of the box. Any natural scientist can tell you what the spider silk protein is, what are its properties and how it functions. But a synthetic Biologist takes this information and transforms it into something new. **A product, a design, a methodology that can benefit society in ways traditional engineering disciplines cannot**. Through synthetic biology, many problems of modern life that were seemingly impossible to solve have been met with a new lens that can **provide new solutions to old problems**.

Consider the case of attenuated vaccines. Attenuated vaccines use a **live but weakened pathogen** to induce both a cellular and humoral response within the patient (Mak & Saunders, 2006). These vaccines often **confer long term resistance** to the target pathogen however they are often difficult to manufacture. A common method of attenuated vaccine manufacturing is a process called **serial culture**. During serial culture, also called serial passaging pathogens are **cultured in various media that progressively degrade the efficacy of the pathogen** resulting in a culture that contains extremely weakened pathogens (Somerville et al., 2002). This process of attenuation can take years and it **poses the risk of not sufficiently weakening the pathogen** resulting in exposure of patients to a live pathogen.

This problem is being addressed by a wide variety of disciplines including the rising field of synthetic biology. Alongside the development of next generation sequencing, researchers in the field of vaccinology have developed a novel method of creating vaccines known as codon deoptimized attenuation. Here, we will explore the concept of codon deoptimized attenuation and understand not only its relevance to vaccinology but appreciate this technology as a part of a greater push of synthetic biology in therapeutics.



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The biased genome

Amino acids are encoded by trios of nucleotides called codons. There are 20 amino acids that need to be coded for meaning that aside from methionine and tryptophan, **every amino acid is encoded for by more than one codon**. This is why the codon table is described as degenerate, because there are more codons available than there are amino acids (Osterrieder & Kunec, 2018). Most organisms do not use each codon equally and many **prefer to use certain codons over others**. As an example, Escherichia coli will preferentially encode Leucine with the codon CTG 47% of the time while other codons coding for the same sequence such as CTT, CTA, CTC, TTA and TTC are less frequent. While not fully clear why this phenomena occurs it is believed to be **due to varying abundance of tRNA in cells** resulting in certain anticodons being more abundant than others (Ikemura, 1985).

Codon optimization is a common practice in synthetic biology due to the many recombinant genes expressed in the field. In this process, a sequence from one organism has its codons **converted from the codon bias of the original organism to the codon preference of another**. As an example, in S.cerevisiae the most frequently used codon for Leucine is TTG at 0.29. If someone wanted to express a gene from E.coli in S.cerevisiae they need to change all Leucine codons in E.coli to the optimal codon used by S.cerevisiae to ensure maximal production of a gene product.

Lack of codon optimization can result in poor levels of expression as the tRNA required for the unoptimized codon are in lesser abundance (Mauro & Chappell, 2014).

While high efficiency is ideal for protein synthesis, synthetic biologists have identified that codon manipulation in the opposite direction can also be useful in vaccine development. The rationale behind codon deoptimization being that if viruses have their codons modified to their own rare codons or the rare codons of a host, the **resultant virus would have decreased expression levels resulting in attenuation** (Osterrieder & Kunec, 2018).

Steps of codon deoptimization

With the advent of rapid sequencing technology, the **discovery of a new pathogen is often swiftly followed by a full sequencing of its genome** so that researchers can develop therapeutics against it. As an example, the COVID-19 RNA sequence was sequenced only months after first detection in Wuhan China (Wu et al., 2020). Once this data is released, scientists are in a rapid race to develop drugs and vaccines to combat the pathogen.

TREADING WATER

WRITTEN BY KALYAN GHADIYARAM
EDITED BY ALEXI MERY
DESIGNED BY WING YIN LIU

Codon deoptimized attenuated vaccines rely on this information heavily as these vaccines are produced from sequences, not from purified virus particles. Following sequencing it is important to identify relevant coding regions that are to be deoptimized. Codon deoptimization and attenuated viral vaccines in general run **a balancing game between weakening the pathogen sufficiently to not harm the host and to be close enough to the pathogenic strain to elicit an immune response**. Additionally, the number of codons deoptimized needs to also be considered. Coleman et al. (2008) deoptimized nine degenerate amino acids within the Sabin type 2 poliovirus in the capsid part of the viral genome. Interestingly, attenuation in this case did not result in decreased protein expression when compared to the wild type. Instead, what was discovered was that the **amount of viral RNA** being produced in the HeLa cells sued as a host was **decreased by roughly 3 folds**.

Newer developments in codon deoptimization have additionally also found that **codon pair bias** is a phenomenon that can be used to create attenuated vaccines. Research has found for example that not only are codons biased towards certain sequences but even **pairs of codons can be biased**. As an example, the Ala-Glu sequence in humans is preferentially encoded by GCAGAG compared to GCCGAA by a factor of 7 (Coleman et al., 2008). The origin of codon pair bias is unknown but we are aware that the mechanism behind this phenomenon is unrelated to single codon bias (Mueller et al., 2010).

In another study done on poliovirus, researchers investigated the effect of codon pair deoptimization on the viability of poliovirus. Similar to Coleman et al.(2008), Mueller et al. (2010) modified the virus at the capsid region. They created two types of polioviruses called PV-MIN and PV-MAX. The PV-MIN virus was modified to have codon pairs that were **under-represented in the human genome** while the PV-MAC virus was modified to have codon pairs that were **over-represented in the human genome**. These virus constructs were then used to infect HeLa cells to investigate viability. Results showed that while **PV-MAX showed cytopathic effects** within 24 hours of infection, **PV-MIN produced no cytopathic effects** 96 hours after infection and even after four blind passages of the supernatant from transfected cells suggesting that **PV-MIN has been successfully attenuated.**

Codon pair deoptimization for an Influenza Vaccine

Flu season is a common phenomenon that causes many people inconvenient symptoms such as coughing, sore throat and lethargy. However, in the US alone, Influenza causes over 20 000 deaths and 400 000 hospitalizations per year (Luckhaupt et al., 2012). **Current vaccines** use killed virus particles which are **excellent at inducing antibody production**. However, more and more research shows that **cell mediated responses** such as cytotoxic T-cells that target virally infected cells also **play a crucial role in dealing with the influenza virus** (Rimmelzwaan, Fouchier, & Osterhaus, 2007). This is where current vaccines prove less effective and where **live attenuated vaccines developed by codon deoptimization can shine.**

In one study conducted by Nogales et al. (2014), researchers attempted to use codon optimization on influenza A. Due to the large demand for information on the influenza virus, databases such as the Influenza Virus Resource database allow free access to nucleotide and protein sequences of a wide variety of influenza viruses to researchers worldwide. Using known sequence information on the Influenza A virus **researchers identified the NS protein as a potential attenuation target** using codon deoptimization. The NS gene codes for two proteins of independent function. One of the protein products inhibits Interferron-1 function while the second product nuclear export protein (NEP) is involved in the export of ribonucleoproteins (Nogales et al., 2014). Both these roles are **crucial to the pathogenicity** of the influenza virus and are therefore **ideal targets for attenuation**. Researchers developed three strains for investigation, one strain with NS1 deoptimized, one strain with SEP deoptimized and one where the entire NS coding region was deoptimized.

In the kidney cell line MDCK, researchers found that **all three strains had reduced protein expression of their codon deoptimized sequences**. They noted that NEP had more protein expressed than other strains, but this was explained as due to a reduced number of codons deoptimized in the NEP sequences at 4.25% compared to the NS1 codon deoptimized strain as an example at 27.76% of codons. Results also showed **a potent immunization capacity in mice**.

Notably, because NS1 plays a crucial role in Interferron-1 antagonism, inhibition of this gene **provides an advantage for immune cells which use IFN-1 during the early stages of infection**. This was further confirmed by comparing the wild type NS strain to the NS codon optimized strain with the latter showing increased IFN-1 expression.

Work like this and other could hopefully make codon deoptimized vaccines a staple of modern healthcare. The major limiting step of codon deoptimized attenuated vaccines is that the **molecular mechanism that causes codon and codon pair deoptimization to occur remains unknown** (Coleman et al., 2008). In a world where anti-vax rhetoric remains prevalent, it is important to be fully aware of what goes into a vaccine. In a field such as synthetic biology, every step is part of a greater engineering cycle and without understanding of the fundamental principles behind a therapeutic, it will have **difficulty making it to clinical trials**.

Concluding remarks

Codon deoptimization provides a **fast and efficient way to produce attenuated vaccines**. While not fully understood, the leaps it has made in providing a quick and efficient method of developing vaccines makes it a continued field of investigation. While still in its early stages, **codon and codon pair deoptimization** can hopefully some day make it big in the field of vaccine science with the next steps of research being to **better understand its mechanism of function.**

SHALLOW DIVE

WRITTEN BY ALEX EPSHTEIN
EDITED BY NICOLA ALLEN
DESIGNED BY DORIS YU

The History of E.Coli and How Humans Harnessed its Power

The toughness, variety, wide-ranging hardiness, and ease of management of Escherichia coli (E. coli) makes it the **most widely studied and well-understood organism** on the planet. Although mainly studied as a model organism extracted from natural history, E. coli is not just used for microbial experiments. Rather, it is a highly diverse organism with a complex and multifaceted niche in the wild. The study of E. coli in the wild reveals more about the presence of it in the environment and highlights its importance in contributing to **global diversity and genomic evolution**, as well as its role in the **human microbiota and disease**. These findings have revealed aspects of its biology and ecology that pose far-reaching questions, illustrating how an appreciation of E. coli's natural history can expand its value as a model organism, potentially changing the future and fate of our planet forever.

However, E. coli's presence in the environment has also been a **cause for concern**. E. coli has been found to be a major cause of diarrhoeal diseases, peritonitis, colitis, bacteraemia, infant mortality, and urinary tract infections that worldwide **cost billions of dollars to treat** and **kill approximately 2 million people each year**. Some strains may cause cancer, with pathogenic strains producing virulence factors causing illness in even the healthiest host. Its power to multiply at rapid speeds, but also have the ability to debilitate and revoke life, can have devastating consequences as it assumes control over all aspects of life. **But what if humans learnt to harness E. coli's 'invisible' power?**

The first scientist to isolate E. coli was the German physician **Theodor Escherich**. Escherich was devoted to the improvement of child care and reducing the high levels of infant mortality that were prevalent during the 1880s. When he moved to Munich, he began a series of studies investigating the effects of gut microbiota on gastrointestinal physiology and pathology. Escherich devoted himself to bacteriology, introducing new methods in

evaluating the faeces of infants, presenting his research in 1885 to the Society of Morphology and Physiology, in which he described the morphology and properties of a bacterial population that he had observed in the lower sections of the gut. After 18 months of intensive research, Escherich published his 'habilitation' thesis entitled "**The intestinal bacteria of neonates and their relationship to the physiology of digestion**", a body of work that established him as one of the **leading bacteriologists** of his era and a **pioneer of microbiological research**.

E. coli has come a long way since first appearing in Escherich's lab. Now, scientists use E. coli as a **foundation for the majority of their microbiological research**. If you look far enough, you can find E. coli in almost everything, from everyday medication or food supplements, to buildings and biofuels. Scientists now use E. coli to store DNA sequences from other organisms, to produce proteins, and to even test protein function. Advancements such as these largely contributed to the rise of new fields within biology, such as synthetic biology and biotechnology which specialise in redesigning organisms for useful purposes by engineering them to have new abilities.

Synthetic biology allows us to harness the power of natural organisms. It is **reframing** how we approach biotechnological development by uncovering new engineering principles to solve existing challenges. **New drug delivery technologies** will revolutionise how we fight disease. he rise of synthetic biology offers **completely new perspectives** to **healthcare**, treating a range of diseases with cell-free diagnostic approaches in the hopes of identifying diseases and environmental contaminants. This would introduce **new bio-manufacturing technologies** that will help us sustainably supply the world with countless products. Due to the vast amount of research on E. coli and its rapid reproducing power, it is commonly used to manipulate its genome to allow researchers to see progress as fast as overnight. In recent years, thanks to synthetic biology, E. coli has been used to produce **cancer-fighting drugs** as well as make **essential antibiotics**, and even manufacture **insulin**.

However, many questions are yet to be answered. How does E. coli adapt to non-host environments? What role does it play in non-host communities? Just how fluid is the E. coli genome and how do environmental and ecological conditions affect this fluidity? How is E. coli adapting to changes in the human diet and lifestyle? These are all questions microbiologists hope to answer in the upcoming years.

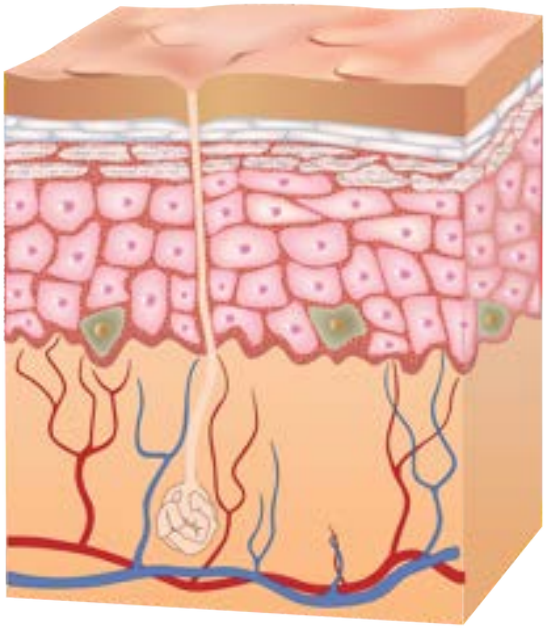
Nevertheless, E. coli continues to provide the **backbone to so much biological research**, it is sure to be a component of future research too. With biotechnologists and synthetic biologists dedicated to studying the bacterium, more attention is being placed on the construction of biosensors or microbial cell factories that may have invaluable applications in healthcare, the environment, and industry. All these discoveries may potentially be used to prevent future disasters, such as disease outbreaks as well as minimise the impacts of climate change and disease outbreaks.

References

TREADING WATER

The Future of Skin Grafts

WRITTEN BY HANNAH SCHICK
EDITED & DESIGNED BY OLIVERA MITEVSKA



The skin can be damaged by a range of problems, such as cancer, skin burns, and other **dermatological diseases** (Swaim, 1990). Replacing the damaged skin is necessary because the skin performs many **beneficial functions**; it protects the body from fluid loss, supports temperature regulation, and helps prevent disease-causing viruses or bacteria from entering the body (Intermountain Healthcare, 2022). **Skin grafts** can restore both the function and appearance of damaged or missing skin. The **grafting process** traditionally includes surgical reconstruction of the skin, but new promising techniques are on the horizon.

With over **160,000** skin grafts being performed annually in nearly 1 out of 3 burn hospitalizations, (Serebrakian, 2018) the skin graft market size is projected to reach **$1.34 billion** by 2028 (GlobeNewswire, n.d.).

The need for skin grafts, coupled with the market's predicted growth, depicts the **importance** of novel methods of skin grafting in the medical field.

One of the main **problems** surrounding surgical skin grafts is the lack of an **autonomous blood supply**, which is necessary for the graft to develop vascularisation with the area of application and 'take' to the skin. In addition, adherence between the graft and recipient is key to keeping the graft in place. These properties of **skin graft application** suggest it's a delicate process and therefore requires advancements to create the ultimate skin graft to correct these issues (Andreassi, 2005).

The development of more effective skin grafts is rapidly growing in the world of synthetic biology. In particular, **genetic engineering** is used to improve the properties of existing grafts such as avoiding rejection and creating a more binding graft. In 2020, the world saw the first application of a genetically modified, live-cell pig **xenograft*** surgery (American Association for the Advancement of Science, 2019). In the past, pig xenografts haven't been as successful in transplantation due to the differing antibodies on the pig ve-

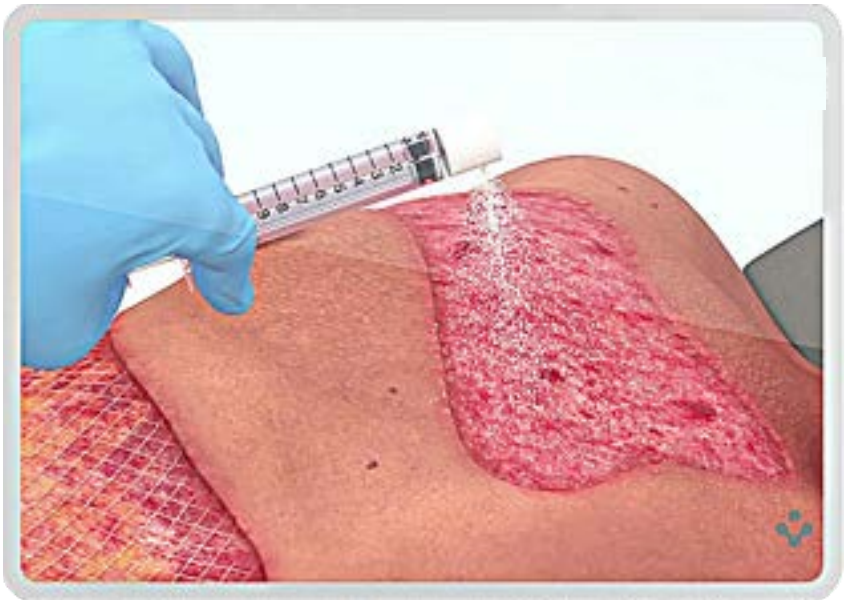


Figure 1. ReCell device being used to treat a burn

-rsus human skin which cause rejection when transplanted onto human skin. However, through genetic manipulation, the expression of these surface **antibodies** that cause an unwanted immune response can be eliminated or suppressed to allow for the pig skin to be grafted, remain protected and **heal** properly (Yamamoto, 2018).

Synthetic biology is also being used to create **bacterial adhesins** for skin grafts as there are problems surrounding the adhesion of surgical grafts to damaged skin of patients. These problems occur mainly due to the lack of **'sticky' molecules** in skin grafts. Bacteria produce adhesins, a type of protein which allows them to stick to human cell surfaces, e.g., in the airways. The University of Sheffield conducted a project to genetically engineer bacteria to produce adhesins in the form of **'sticky flagella'** to bind to type 1 collagen in human skin to help grafts bind strongly to the wound, so that vascular remodeling and **restored barrier function** can occur. With the success of this project, further research and funding is taking place with an end goal of implementing this synthetic biology approach to developing novel skin grafts into the **industry**, with many future applications for surgical materials relating to adhesion (University of Sheffield, 2014).

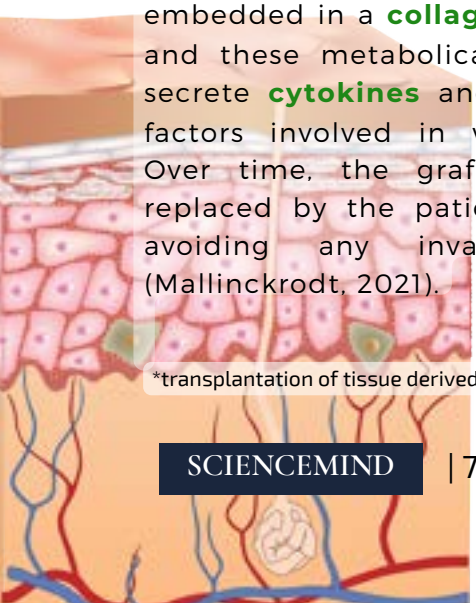
In general, genetically engineering skin cells for graft applications greatly reduces the risk of rejection and promotes **vascularisation**. Culturing stem cells and genetically engineering them to carry functional genes lost in certain skin conditions, can not only act as a **barrier** for the skin, like traditional grafts, but potentially have regenerative properties (Arney, 2018).

Currently, there is a range of techniques emerging used to treat skin wounds that have **regenerative** capacities, and sometimes, entirely without the need for a skin graft. A company called Avita Medical has recently developed a device called **ReCell** (Figure 1) that harvests a sample of a patient's skin and produces a suspension of **spray-on skin** without the need for a graft. The suspension consists of keratinocytes, fibroblasts and melanocytes, all cells

Figure 2. StrataGraft

that stimulate healing throughout the **wound bed**, and is prepared and applied in as little as 30 minutes. Approved by the U.S. Food and Drug Administration (FDA) in September 2018, this system has been successful for treating acute partial-thickness thermal burn wounds, in combination with **meshed autografting** (AVITA Medical, 2021).

Additionally, a company called Mallinckrodt Pharmaceuticals has developed a graft called **StrataGraft** (Figure 2), which unlike other methods of skin graft procedures, does not require the harvesting of patients' own skin cells. The graft is a layer of human dermal fibroblasts embedded in a **collagen-rich matrix**, and these metabolically active cells secrete **cytokines** and other growth factors involved in wound healing. Over time, the graft is eventually replaced by the patient's own cells, avoiding any invasive surgeries (Mallinckrodt, 2021).



*transplantation of tissue derived from a different species to the host

Lastly, **3D skin bioprinting** is another new technique emerging for skin replacement. It involves producing a layer-by-layer deposition of skin cells and **scaffolding materials** onto the patient. This will allow accurate placement of the cells and enable them to behave like natural skin to replace the damaged skin. However, for this to be clinically useful, some **genetic engineering** would be required to allow the vasculature to be accepted and integrated into the host's body (Varkey, 2019).

Overall, there is an array of exciting, newly emerging techniques that could create a **paradigm shift** in the world of skin grafts using synthetic biology, genetic manipulation and new medical devices creating less invasive and more efficient skin regeneration procedures. As the medical industry grows, it is likely that these emerging techniques will conquer traditional methods of **skin grafting.**

References

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DESIGNED BY YUSRA-AINA CHOUDHURY

SHALLOW DIVE

CAN ROBOTS FEEL PAIN?

The Science Behind Artificial Skin

Neurons firing electrical impulses to stimulate a reflex action is what arguably makes us human. Now, however, robots can react to **external stimuli** much like you and I. Recent scientific breakthroughs have showcased the ability of robots to react the same way humans do, sensing pain to external stimuli through various methods of machine learning. Through the development of biomaterials to create **human-like flesh** in combination with different sensors that make up the robots brain, scientists have birthed the new generation of bots, soft robots and electronic skin **(e-skin)**.

ROBOTIC FLESH

When thinking of robots, we often think of machines made from hard metal and a variety of polymers connected to copper wires. Scientists at **Wei Gao lab** at the **California Institute of Technology** were able to divert this stereotype and have developed a robotic hand that mimics the feel of soft human flesh. Through the use of an inkjet printer, they have printed a **gelatinous hydrogel** that consists of sensors to allow the robot to feel its surroundings.

The sensors within the **hydrogel are printed** onto the material much like ink would be on a piece of paper. Mostly consisting of a **base of silver nanowires, carbon and polyimide along with useful sensing nano-materials** embedded within, the sensors serve a purpose of detecting substances that are of potential danger to human health. In an article written about this discovery, Gao describes the idea behind printing the materials.

"Inkjet printing has this cartridge that ejects droplets, and those droplets are an ink solution, but they could be a solution that we develop instead of regular ink."

Purposefully, using a printer to produce the **human-robotic hand** allows for a faster and low-cost method of mass production. Thus, it allows for an efficient method of designing and integrating new sensors to detect chemicals such as **TNT and pathogens**.

Even though definite progress has been seen in the field of research of creating materials that allows for robots to adopt the soft flesh of human skin, the most notable progression is seen in the technology of each robot's software.

HOW ARE ROBOTS BEHAVING LIKE HUMANS?

Humans are powered by a reinforcement and punishment system. It is just simply how the human brain learns what to do and what not to do. Robots are now able to do the same.

For a machine to learn, it has to have an input and an output. Data needs to be processed in order for the machine to make a decision. Traditional methods of developing e-skin focus on pressure sensors that are then sent to a computer to process. This not only reduces reaction time, but also reduces the ability of the machine to make conscious judgments.

Another team known as the **Bendable Electronics and Sensing Technologies (BEST) at the University of Glasgow, led by Professor Ravinder Dahiya**, developed a robotic hand that allows the robot to get in touch with their feelings.

Bringing the robotic hand to life, they have mimicked the human peripheral nervous system (PNS) consisting of **168 zinc oxide synaptic transistor nanowires** printed onto flexible plastic, connected to an array of sensors and transmitters on the palm of the robotic hand.

Have you ever wondered why when touching something hot, we immediately take our hand away? In humans, skin contact triggers our PNS to begin processing information, filtering out unnecessary data and sending only relevant signals to the brain. The process of eliminating sensory data creates an increase in efficient communication channels within the body.

The process of the PNS is exactly what the BEST team implemented in their robots' software. When the robotic sensors come in contact with external stimuli, the sensors respond via a change in electrical resistance - a light touch registers a smaller change of electrical resistance, while a hard touch generates a larger change.

However, the ability of the robot to sense pain is done by connecting a circuit onto the surface skin of the bot, allowing associative learning to take place. **Long-term memory** is stored within the robot by generating an output of **voltage spikes** (whose frequency depends on the pressure of the object pressing onto the skin) stimulating pain. The level of pain is detected by setting a threshold for the voltage, allowing the hand to rapidly react when a sharp object is in contact with the skin of the bot.

By replicating how the human nervous system processes information, robots can now learn to move away from situations of distress, behaving the same way a human would.

Future Implications Of Sensing Robots

Even though the idea of robots feeling pain might be eerie to some, we have officially tapped into the world of human robots. Arguably, this opens the potential to improve various aspects of healthcare and manufacturing.

We have yet to perfect prosthetic limbs, however, with the grand scientific breakthrough of robotic feelings, revolutionising the **manufacturing of prosthetic limbs** to feel pain might give a new perspective to those who require the wear of prosthetics.

In an article written about the BEST's team discovery, **professor Dhayia** mentioned how impactful their discovery would be in the future.

"In the future, this research could be the basis for a more advanced electronic skin which enables robots capable of exploring and interacting with the world in new ways, or building prosthetic limbs which are capable of near-human levels of **touch sensitivity.**"

Not to mention, through robotic feelings we are able to investigate new methods of testing such as examining new pathogens and how the human system reacts to harmful organisms. This can tap into mechanisms that may revolutionise drug discovery and the way scientists view pathogens and their side effects. As well as testing new manufacturing goods such as explosives and objects that may be harmful to test on humans which was one of the objectives of the Wei Gao Lab.

Does this mean robots are becoming more human-like? We are far from creating robots that exactly mimic human systems. Despite the fact that life-sized robots that look and act like humans exist, we still haven't developed a replica that breathes. Soft robots and e-skin technologies are still at its early stages and have yet to re-invent testing mechanisms during manufacturing and healthcare with the objective of improving human health and preventing injury to society.

References

WRITTEN BY ANMOYUL MOHON
EDITED BY IRIS ZIELER
DESIGNED BY YUSRA-AINA CHOUDHURY

TREADING WATER

DEBUNKING THE MYTHS ABOUT CREATINE

Both athletes and recreational non-athletes are constantly looking for competitive advantages in sports and exercise to enhance their health and maximize physical performance. It is important to consider, however, that many other factors impact performance, including smoking, psychological stress, and physical and mental relaxation initiatives (yoga, meditation etc.).

The **use of creatine** as a dietary supplement to increase muscle mass, performance, and recovery gained popularity in the **early 1990s**. Indeed, creatine is one of the most popular sports dietary supplements on the market, with more than **$400 million** in annual sales. Nevertheless, despite its popularity, there are still uncertainties regarding dosage, how it affects athletic performance, and safety.

Despite a lot of studies and peer-reviewed publications a lot of speculation about creatine and its effects is still seen today. In this article, we will cover the nature and desired effects of creatine before going over some of the myths including whether creatine leads to water retention, whether it presents safety concerns, such as **kidney damage/renal dysfunction**, and finally, whether or not it can be considered as an anabolic steroid.

WHAT IS CREATINE AND DOSING?

Creatine **(methyl guanidine-acetic acid)** is a nitrogenous organic compound found in muscle and is available in the diet through the consumption of milk, red and white meat, fish, and mollusks. Although cooking time, meat type, and muscle site all affect creatinine values after consumption, a typical carnivorous diet provides 1 to 2 g of creatine per day. Additionally, the liver and kidneys synthesise around 1g of creatine per day. Depending on the type of muscle fiber and muscle bulk, a young man weighing around **70 kg has a creatine pool between 120 and 140 g**, with 95% of it stored in skeletal muscle.

As a dietary supplement, creatine is marketed as creatine monohydrate or in combination with **phosphorus**. This mimics the form it is found as in skeletal muscle, where two-thirds of it is phosphorylated and the remaining third is free creatine. Skeletal muscle contractions require the energy substrate creatine.

Creatine supplementation in resistance training shows the majority of musculoskeletal and performance benefits in older adults. It also appears safe for short- and long-term consumption by healthy males and females, athletes and recreational fitness enthusiasts, as well as in younger and older individuals. As phosphocreatine is the immediate energy substrate for contracting muscle, its supplementation increases resting phosphocreatine and free carnitine levels in muscles, thus delaying the onset of fatigue thereby enhancing performance.

The recommendations regarding creatine dosing supplementation as an ergogenic aid are varied. While many studies have reported promising results with building lean muscle mass or improvements in "quick burst" athletic performance, there are no firm guidelines regarding a supplementation regimen.

Indeed, **Hall and Trojian advise 0.03 g per kg per day** as a maintenance dose, for **4 to 6 weeks** on average, based on multiple reviewed studies. These recommendations have been increased up to 0.1g per kg per day by others however, maintenance use of creatine has ranged from 28 days to 10 weeks. Most commercially available creatine supplements are packaged to contain between 2 and 5 grams of creatine per dose. While some of these supplements recommend once-daily dosing, many appear intentionally vague regarding recommended frequency or duration of dosing. This is likely due to the fact that comparing studies shows no apparent **dose-dependency** of creatine, leaving the industry with an approximate guess at best.

Creatine 'loading' is defined as supplementing with oral creatine for 5–7 days with a dosage of 20–25 g/day, often divided into smaller doses throughout the day (e.g., four to five, 5 g servings/day). Creatine 'loading' may also be prescribed relative to body mass, for example, 0.3 g/kg/d for 5-7 days (i.e., 21 g/day for a 70 kg individual). The 'loading' phase of creatine supplementation is followed by a daily 'maintenance' phase often ranging from daily 3–5 g servings/day. (figure 1)

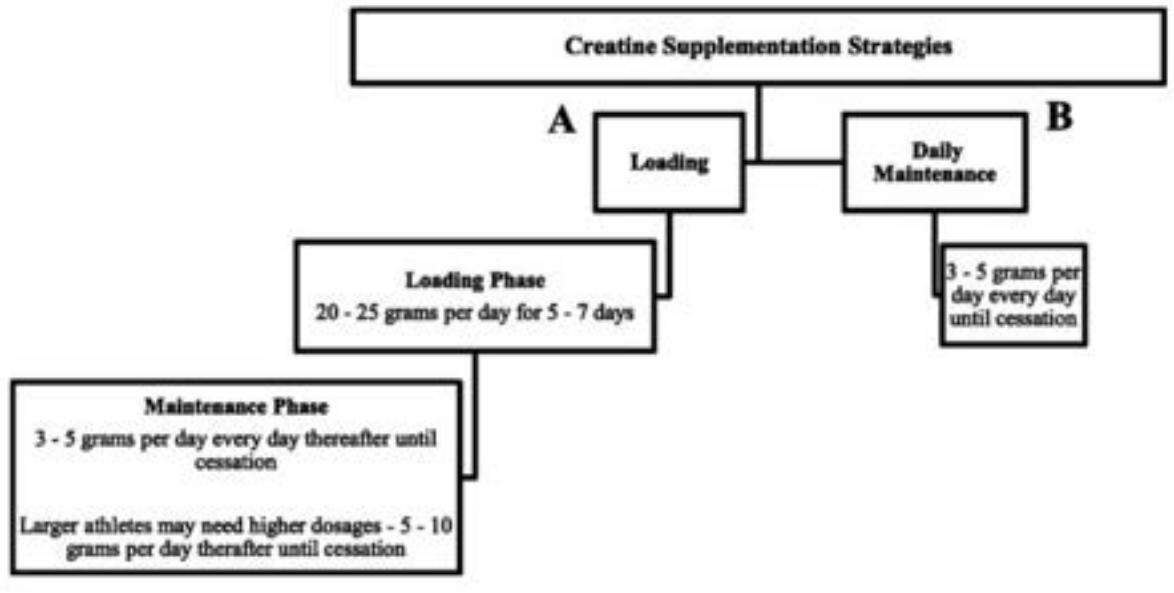

Fig.1: Creatine supplementation strategies. (Loading dose of creatine is often used prior to the implementation of a daily maintenance dose).

MECHANISM OF ACTION, EFFICACY IN SPORT PERFORMANCE AND RECOVERY

Phosphocreatine serves as a source of phosphate to produce adenosine triphosphate (ATP) from adenosine diphosphate (ADP). Skeletal muscle cells store enough ATP and phosphocreatine for approximately 10 seconds of high-intensity activity; short-term creatine supplementation leads to a total creatine increase of 10% to 30%, with phosphocreatine stores increasing by 10% to 40%.

Creatine's ability to increase various parameters of acute exercise performance is well documented. A review by **Kreider in 2003** concluded that approximately 70% of these studies reported an improvement in some aspect of exercise performance. The magnitude of the increase in performance is dependent on a large number of variables, including the dosing regimen, training status of the athlete, and any one of a number of acute exercise variables (intensity of exercise, duration of effort, etc.).

Moreover, an overview of this literature reveals that performance increases of 10%–15% are typically observed. More specifically, 5%–15% improvements in maximal power and strength, anaerobic capacity, and work performance during repetitive sprint performance are commonly reported, whereas improvements in single-effort sprint performance have been indicated to range from 1%–5% improvements. This is because increased creatine stores is especially advantageous in the phosphocreatine resynthesis after its depletion by an intensive bout of exercise. As such, stores can be refilled more rapidly, enhancing performance in repeated bouts of maximal power output.

Although no consistent reports indicate that supplementation with creatine may have an ergolytic or performance-decreasing response, a large number of studies report an increase in body mass of 1–2 kg during the first week of loading, which may **hinder performance**, depending on the type of athlete and the phase of training.

Harris et al were the first to document increased muscle creatine concentrations of 20% with creatine supplementation in the form of creatine monohydrate. Creatine supplementation increases lean body mass as well as strength, power, and efficacy in short-duration, high-intensity exercises.1 These ergogenic effects have been studied extensively in the weight room and laboratory, with limited studies in active gameplay scenarios. **A meta-analysis from 2003** including 100 studies demonstrated significant improvements in laboratory-based exercise but did not show improvements in sports-specific activities after short-term creatine supplementation.

One of the ergogenic effects of creatine supplementation is increased body mass. A meta-analysis showed that approximately 64% of studies measuring body mass and/or body composition noted a statistically significant increase in lean body mass due to creatine supplementation. The increases in body mass were thought to be the result of increased intracellular water related to fluid shifts due to the osmotic properties of creatine.1,4,36 Increased body mass has been noted in those using a creatine supplement without participation in an associated exercise program. However, taking creatine in conjunction with a resistance training program yielded greater increases in body mass, suggesting a role in muscle hypertrophy

For example, when it comes to pure Strength and Power, creatine supplementation has been demonstrated to increase muscular strength and/or power, and these findings have included trained and untrained men and women.

As measured by 1-repetition maximum, muscular power, number of repetitions, muscular endurance, speed, and total force.1,4,40-44,47,48 Strength gains after 28 days between groups taking creatine alone, creatine plus resistance training, and placebo plus resistance training showed all groups significantly increased **(P < 0.01)** bench and leg press muscular strength, with the creatine plus resistance training group improving the significantly more than the group taking creatine alone.2 In meta-analyses of creatine supplementation on upper and lower extremity performance, increased strength performance related to creatine supplementation was noted for both the upper and lower extremity. There was an improved performance with creatine supplementation in conjunction with a resistance training program, especially evident in those with no previous training history **(defined as exercising less than 3 h/wk).** Changes in performance were independent of age, sex, supplement dosage, and supplement duration. The meta-analysis focusing on upper extremity response to supplementation displayed the most significant strength increases, mainly at the pectoralis muscles (major and minor), with performances in bench press increasing by approximately 5.3% with creatine supplementation. Other studies have demonstrated similar improvements in bench-press performance.

The primary mechanism behind these ergogenic outcomes for creatine supplementation appears to be attributable, in part, to increases in intramuscular phosphocreatine concentrations. Due to its potential not only to enhance strength and power output but also to expedite recovery from intense intermittent exercise, creatine supplementation has been shown to allow for increased volumes of work and increased work output during resistance training and sprints, which may then translate into greater strength gains.

For many athletes and coaches, the **impact of creatine supplementation** on sports performance is the most important consideration. It is well established that creatine supplementation leads to increased muscle intramuscular phosphocreatine content, and thus accelerated ATP resynthesis, and enhanced performance in short-duration, predominantly anaerobic intermittent exercise. As a result of these observed benefits, it has been suggested that creatine supplementation could translate to enhanced on-field performance for competitive athletes. The ever-changing nature of sports in terms of intensity, distances covered, and duration makes the replication of sports performance difficult.

Other outcomes of creatine supplementation have been purported to impact endurance training and performance. For example, **studies have demonstrated** that adding creatine to carbohydrates or carbohydrates + protein supplementation may help promote greater glycogen storage. The proposed mechanism is through **upregulation of GLUT4,** the glucose transporter found in skeletal muscle. In many endurance activities, the intensity and duration of training and competition cause drastic reductions in hepatic and intramuscular glycogen levels, such that enhancing glycogen storage by adding creatine to carbohydrates and carbohydrates + protein feeding may prove beneficial.

Nevertheless, results have been inconsistent overall with regard to an individual athlete's response to creatine supplementation. This may in fact be due to the preloading of creatine. Athletes with a higher baseline level of creatine before supplementation are less likely to derive benefits than an athlete with a low baseline level of creatine. This likely explains why some athletes appear to be "responders" to creatine supplementation while others are "nonresponders."

Aside from overt improvements in the performance of single bouts of maximal efforts, creatine demonstrates a role in **enhancing recovery.** The term recovery is often contextual in nature and typically pertains to either physiological, subjective, or performance-based parameters. In this respect, creatine appears to positively influence recovery in regard to physical performance following bouts of intense activit and has been shown to enhance recovery during bouts of intermittent activity, sustaining maximal performance across multiple bouts of exercise.

In addition, creatine supplementation may also reduce the post-exercise inflammatory response, thereby attenuating markers of muscle damage and soreness in the hours and days following bouts of exercise-induced muscle damage. This, again, is related to the more efficient ATP generation, resulting in enhanced regenerative capacity following exercise-induced muscle damage. Finally, creatine may possibly provide synergistic benefits during the post-injury rehabilitation period and a certain efficacy as a therapeutic intervention following an injury or during periods of limb immobilization .

PERSISTING SAFETY CONCERN

Short-term use of creatine is considered safe, although caution should be advised as the number of long-term studies is limited. **The International Society of Sports Nutrition** notes that "there is no scientific evidence that the short- or long-term use of creatine monohydrate has any detrimental effects on otherwise healthy individuals." They go on to say that "supplementation in young athletes is acceptable and may provide a nutritional alternative to potentially dangerous anabolic drugs."

Many theories regarding the **adverse effects** of creatine have been proposed, including the potential for renal damage, hepatic injury, and difficulty maintaining hydration. There have been theoretical concerns regarding the potential effects of creatine supplementation on renal function.

In skeletal muscle, creatine and PCr are degraded non-enzymatically to creatinine, which is exported to the blood and excreted in the urine. Healthy kidneys filter creatinine, which would otherwise increase in the blood. Therefore, blood creatinine levels can be used as a marker of kidney function. However, the amount of creatinine in the blood is related to muscle mass (i.e. males have higher blood creatinine than females) and both dietary creatine and creatinine intake. Both blood and urinary creatinine may be increased by the ingestion of creatine supplementation and creatine-containing foods, such as meat. Creatine is normally not present in urine but can reach very high levels (>10 g/day) during creatine supplementation.

Overall, depending on most research trials investigating the effects of creatine supplementation on kidney/renal function in healthy individuals, it appears not to carry adverse effects when consuming recommended doses of creatine supplements. Interestingly some of the **case studies which reported renal dysfunction** in individuals were all were confounded either by medications, pre-existing kidney disease, concomitant supplement ingestion, or as simply as inappropriate creatine dosages (e.g., 100 X recommended dose), and anabolic-androgenic steroid use.

Moreover, If the link between creatine supplementation and kidney health was valid, there would be an expected increase in kidney damage / renal dysfunction in low risk (i.e. young, physically fit, healthy) individuals **since 1992 after Harris et al.** published their seminal work .

After nearly 30 years of post-marketing surveillance, thousands of exposures, and multiple clinical trials, no such evidence exists.

Creatine is known to cause mild water retention and decreased urinary volume due to its osmotic effect. This may result in temporary weight gain, particularly during the loading phase. Because of the increased intracellular water volume, there is an increased risk of **compartment syndrome**, muscle cramps, dehydration, or heart illness. However, none of these potential adverse reactions have been supported. The safety of creatine supplementation is unknown in children and adolescents.

One of the **biggest misconceptions** about creatine is that it is an anabolic steroid. Anabolic steroids are a synthetic steroid hormone that mimics testosterone, an androgenic hormone that is also produced endogenously within both males and females (2.5 to 11 mg daily and about 0.25mg daily respectfully), especially in response to exercise, and is used in conjunction with resistance training with the intent of enhancing muscle mass and strength due to increases in muscle protein synthesis.

Testosterone (Figure 3) is the key androgen promoting masculine (androgenic) characteristics, as well as maintaining nitrogen balance and facilitating protein synthesis (anabolism). Although both males and females synthesize testosterone, serum concentrations in males (300 to 1000 ng/dL) are significantly greater than in females (15 to 65 ng/dL). While the physiological and performance outcomes of anabolic steroids and creatine can be similar, their mechanisms of action and legal categorization are not. Anabolic steroids are drugs, with a different chemical structure than creatine (figure 2), this is why Creatine cannot be considered an **anabolic steroid** as it has a completely different chemical structure

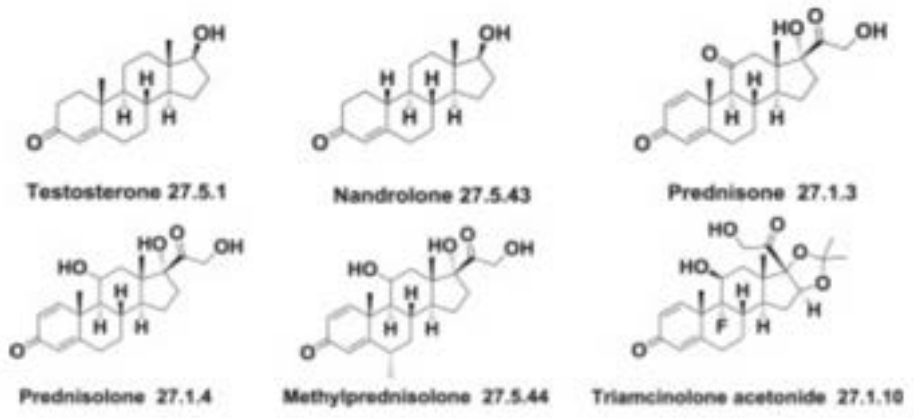

Figure 2; Chemical structure of steroid hormones with three 6-carbon rings and one 5-carbon ring.

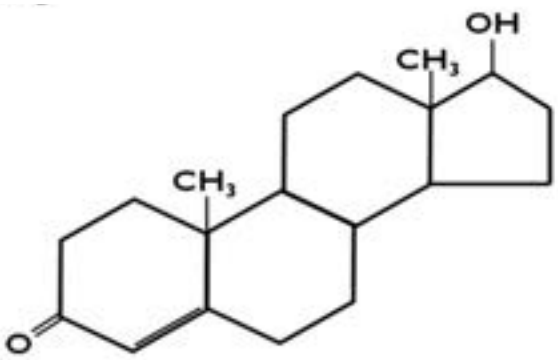

Figure 3 Chemical structure of testosterone.

Figure 4; Chemical structure of creatine

Short-term use of creatine is considered safe and without significant adverse effects, although caution should be advised as the number of **long-term** studies is limited. The safety of creatine supplementation has not been studied enough in children and adolescents. Currently, the scientific literature supports creatine supplementation for increased performance in short-duration, maximal-intensity resistance training. Creatine supplementation intermixed with carbohydrates or carbohydrates and protein appears to be efficacious in increasing intramuscular glycogen storage, although the additional benefits in terms of performance outcomes appear to be nebulous.

However due to the difficulties of reproducing real-life sport-specific simulation in the laboratory, whether or not these creatine supplementation effects do lead to improved performance in the actual field of play remains unknown.

Augmenting the intramuscular creatine stores either by creatine loading or daily supplementation over several days leads to increased concentrations of intramuscular creatine and intramuscular phosphocreatine. Increasing these substrates thus enable a faster and longer-lasting source for anaerobic ATP production, thereby increasing energetic output during activities involving intermittent, high-intensity, short bouts of exercise. Additionally, creatine supplementation shows promise in facilitating recovery following exercise-induced muscle damage and potentially as an aid during post-injury rehabilitation.

References


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