Chronovaccination: the competitive edge in vaccine effectiveness?

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Abstract

Although chronovaccination - controlling the time of day of vaccination (TODV) to enhance protective immunity - has been described as a potential paradigm shift in vaccine immunology[1], unequivocal evidence for clinical benefit outweighing other societal and practical considerations has yet to be established[2]. The current mixed evidence for chronovaccination providing an additional competitive edge to vaccine effectiveness is likely due to complex population level interactions between various circadian, environmental, and sociodemographic factors influencing antibody responses to vaccines. Nonetheless, TODV shows the greatest potential for sub-cohorts of populations, particularly older age, and immunocompromised groups. In these contexts, this review provides a Person, Place and Time consideration of the factors influencing the outcomes of chronovaccination studies.

Plain Language Summary

Researchers have asked whether there is a 'sweet spot' in the day where vaccination is most effective in terms of providing long-term protection. So far, their answers have not been crystal clear, probably due to the different ways in which studies are performed and the daily variability of our immune system responses for populations in our modern 24/7 society. However, scheduling vaccinations focussed on distinct phases of the day, mornings, afternoons, or evenings for example, might be beneficial for groups where a boost in vaccine effectiveness may be especially welcome. This review sets out the key concepts when thinking about how our internal timing mechanism – our circadian clock – might be used to get the most out of vaccinations, similar to how our clocks are harnessed in other areas of society.

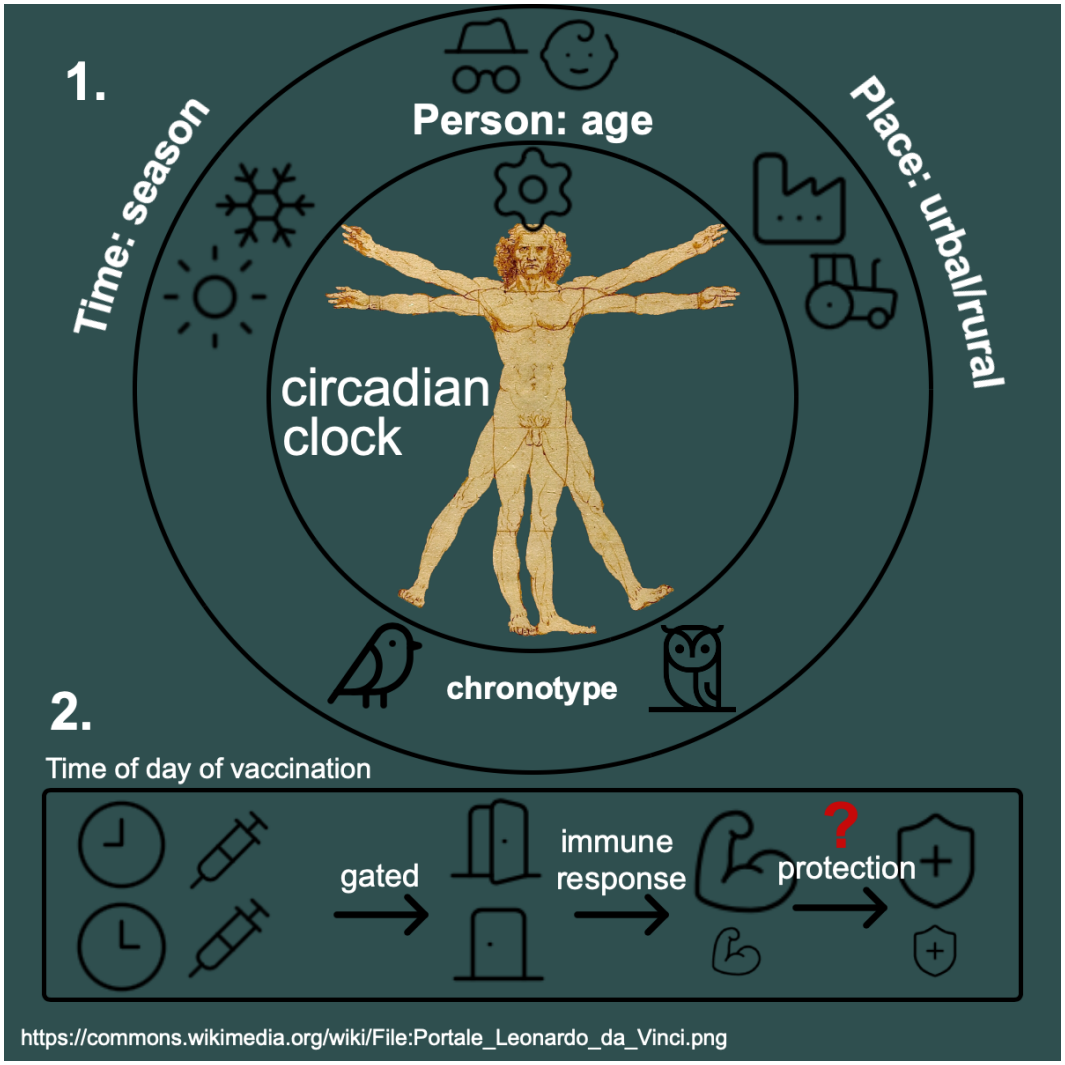

Figure 1: Graphical abstract

1 Introduction

Researchers in epidemiology and public health commonly use three descriptive variables - Person, Place and Time - to look for associations and health determinants explaining health phenomena. This review highlights the key features of the mammalian circadian clock and its role in the daily gating of our immune function, and uses Person, Place and Time to describe the features that impact the close relationship between circadian clocks, their environment, and our immune system. An understanding of these relationships is crucial to our awareness of confounding factors in epidemiological studies addressing the question as to whether daily variations in our immune response extends to our antibody response to vaccination.

1.1 The circadian clock

Whether we realise it or not, our internal timing mechanism, or clock, dictates our daily lives by harmonising our physiology with alterations in our external environment, principally the predicable alterations in light and dark that are hallmarks of life on a revolving planet. Simplistically, the clock is a network of clock genes whose sequential transcription and translation progress over a near 24-hour period.

These daily cycles, often referred to as circadian rhythms (derived from the Latin 'circa diem' or 'about a day'), orchestrate virtually all aspects of our physiology, including sleep-wake cycles, behaviour and locomotor activity, body temperature cycles, cardiovascular and digestive processes, endocrine systems and metabolic and immune functions[3–8]. A copy of the clock is found in most cell types including cells of the immune system[8,9], but precise and robust coordination of circadian rhythmicity is the product of a network of tissue and organ clocks, including a central clock – primarily influenced by light - situated in the suprachiasmatic nucleus (SCN) of the hypothalamus brain region that dictates the overall pace for synchronising peripheral clocks located in virtually all tissues and organs of the body[10].

1.2 Clock plasticity permits entrainment

Clocks exist in Nature where the environment is in a constant state of flux. Over the course of evolution, clocks have developed the capacity to adjust their internal rhythms based on fluctuating environmental variables, and a key consequence of this plasticity is the ability for circadian clocks to be reset daily, or entrained, by these signals, or zeitgebers ('time givers') - notably light, but can be other cues, for example temperature, food availability, hormones[11,12] and even social signals[13]. Entrainment allows the clock to readapt upon temporary desynchronisation, whether this is due to misalignment between an individual's internal circadian phase and their environment, for example when crossing multiple Time Zones ('jetlag'), as a result of abrupt changes to work patterns, such as shift work, or due to social jetlag[14] - the discrepancy between work and free days, between social and biological time. Daylight saving is another example of temporary desynchronisation in which misalignment occurs between endogenous circadian phase and shifted clock time. While the SCN central pacemaker itself adjusts to changes in the environment relatively quickly, the peripheral clocks can take longer to adjust[15,16]. Chronic circadian disruption has serious implications for human health and can increase the risk for the expression and development of neurologic, psychiatric, cardiometabolic, and immune disorders[17,18].

1.3 Clocks, what are they good for?

Broadly speaking biological clocks allow us to adapt to and anticipate temporal changes in the environment. Clocks have evolved over billions of years, possibly in response to the need for single celled eukaryotes to 'escape from light' to avoid the damaging effects of ionising radiation and oxidative stress during cell division[19,20]. At the core of mammalian clockwork are a set of conserved cellular transcription factor proteins operating as interlocked rhythmic transcription-translation feedback loops. The formation, cellular trafficking and degradation of different clock protein complexes throughout this transcription-translation cycle generates the intrinsic nature and stability of the clock, ultimately driving the rhythmic expression of the clock genes themselves as well as 'outputs of the clock' such as clock-controlled genes[9]. Essentially then, the rhythmicity of the core clock radiates out to the physiological outputs of the clock via interaction with clock-controlled genes. The clock therefore does not orchestrate a single output, but rather partitions physiological outputs to appropriate phases of the day. This is beneficial because by timing physiological processes appropriately – or 'gating' these processes – cellular resources can be effectively and efficiently allocated.

Clocks provide a competitive advantage for the organisms they serve. The best demonstration of this is for experiments in plant circadian biology where for plants genetically compromised in the pacing of their clock - and therefore unable to match the light and dark conditions of their environment – survived more poorly than plants able to correctly match the external light-dark cycle[21]. Clocks anticipate predictable changes in the environment (light & dark, warm & cool, for example), rather than reacting to change. For example, in plants again, it has been shown that photosynthetic machinery is assembled daily before the onset of dawn in anticipation of the availability of light so that energy harvesting is optimised[22]. In mammalian

systems the liver and intestine clocks anticipate, rather than react to, mealtimes to coordinate food availability with nutrient processing and energy demand[4,23].

The 'clock as a tool' for optimising performance is increasingly being harnessed in modern life. A good example of this is in the arena of elite sports, often occurring across multiple Time Zones, where adapting training strategies to align with circadian regulation is now viewed as essential. Marginal, yet meaningful, advantages over competition can be gained when clock factors are keyed into training regimes[24]. 'Tools for the clock' are also increasingly apparent, for example the development of non-invasive wearable smart devices for characterising the clock, viewed as crucial for advancing personalised medicine – developments that can be viewed as providing an extra edge or dimension via the design of individualised treatment plans tailored to the specific needs of patients[25,26].

Therefore, clocks themselves inherently provide a competitive edge and, thanks to decades of fundamental research into circadian clock function, we increasingly see clock knowledge harnessed for gaining competitive edges in various societal contexts, many of which are referred to as chronotherapies. Chronovaccination – a vaccination chronotherapy – has the potential to provide a competitive edge in vaccine effectiveness.

1.4 Circadian gating

A useful concept for the utility of circadian clocks is the idea that they gate biological events to particular times of the day[27]. While circadian gating has different meanings depending on the types of physiology or behaviour under investigation, for example whether entrainment to zeitgebers is gated or the amplitude of rhythms are modulated, gating can be simply thought of as time periods when the clock allows or forbids a particular biological process. Chronotherapy harnesses the principles of circadian gating and has been, for some time now, regarded as a beneficial and wide-ranging socio-economic tool, from the timing of the application of agrochemicals[28], to the administration of anti-malaria drugs at specific times of the day[29], to the rhythmic responses to drugs in mammals[30,31]. In the latter case drug timing is optimised to enhance drug efficacy, reduce toxicity and adverse side effects and improve patient outcomes in, for example, cancer treatments[31,32]. A seminal study in 2014 by Zhang *et al.*[30] found that 119 of the WHO's list of essential medicines target a circadian gene, and since many of these drugs have short half-lives (<6 h), the impact of the time of administration on their action is significant[30]. For example, statins are a class of drug that lower cholesterol levels by inhibiting HMGCR (HMG-CoA reductase)[33]. HMGCR is the rate-limiting enzyme in cholesterol biosynthesis and its activity peaks during the night. Statins with short half-lives showed maximal efficacy when taken in the evening (when their target gene was most active)[30]. There are now numerous examples of health conditions where timed drug treatments align with the circadian gated biological target, so called 'clocking the drugs'[31], examples include hypertension, diabetes and anti-inflammation[31,34,35]. Despite this, many in the scientific community still consider 'time-of-day' a neglected variable in fundamental biological and clinical research[35–37]. The lack of circadian enlightenment extends to clinical practice where only 4 of the 50 most prescribed drugs in the United States in 2019 had time-of-day dosing recommendations from the Food and Drug Administration, and the 20th World Health Organization's Model List of Essential Medicines makes no mention of dosing time[38]. There have therefore been calls for time-of-day to be recognised as a key biological variable, similar in status to the ubiquitous sex and age variables, in both clinical studies and clinical practice[35,38,39]. Indeed, chronotherapies that consider time of day may significantly improve clinical trial success and therefore ultimately patient care[39].

1.5 Clock gating of the immune response

Innate and adaptive defences constitute two functionally distinct, yet intertwined, arms of defence against pathogenic invasion or insult, and while the former arm is regarded as a first line of defence with no memory of the challenge, adaptive immunity provides more finely-tuned, specific recognition of foreign particles and the selective expansion of cells prepared to target specific pathogens and the development of immunological memory[40]. Vaccinations utilise the adaptive immune system to generate antibodies towards a target antigen as well as the development of 'immune memory' - the capacity to respond quicker and more effectively when challenged after an initial exposure[41]. The function of the adaptive immune system is regulated by the circadian clock, with the clock contributing to, for example, the 24-h regulation of homeostatic processes underlying maintenance of adaptive immunity, such as lymphocyte (white blood cell) trafficking[40,42,43], where the clock essentially modulates leukocyte migration across the body, effectively gating the number of leukocytes at specific sites throughout the body across the day[44].

It is therefore generally regarded that the circadian clock is a potent regulator of immune function[6–8,40,45], however less clear is why our immunity is gated by the clock? One idea is that since metabolic and immune processes are intricately linked, over the course of evolution these processes have been partitioned[46]. This is evidenced by disruption of clock gene function resulting in a host of metabolic disorders, which are often associated with immune observable traits, or phenotypes[47]. Another idea is that heightened immune sensitivity is an adaptation to recurring environmental challenges (such as time-of-day prominence of pathogens), with the additional benefit of dimming immune responses at times when they are not needed[9], chiming with the idea that the metabolic costs of immune activation are monitored closely by the clock so that immunity-associated metabolic costs are kept in check, in turn, enhancing organismal fitness[45].

There are many examples of the detrimental effects of a disrupted clock on aggravating disease pathology related to our immune system. Employment in rotating shift work is a prime example and has been associated with an increased risk of developing inflammatory diseases such as psoriasis and irritable bowel syndrome[48,49]. It is also well known that chronic inflammatory disease symptoms can show time-of-day variation; for example, asthma, whereby symptoms are exacerbated in the early morning[50], or increased joint stiffness and pain in the early morning with rheumatoid arthritis sufferers[51]. Other studies have linked social jetlag to higher levels of inflammatory markers, and a study of the effects of circadian and sleep rhythm disruptions on immune biomarkers among hospital healthcare professionals working night shifts and rotating day shifts showed that night workers altered pattern expressions of immune cells biomarkers[52] may increase vulnerability to infections and reduce vaccination efficiency. Increased inflammation can weaken the body's defences and make individuals more susceptible to infections and reduce vaccine efficiency[52].

However, while this discussion has focussed on the role of the clock in gating immune function, the influence of sleep on immune function cannot be ignored. Traditionally, clocks, sleep and the immune system have been regarded as separate subjects, but it is increasingly clear the extent of the interconnectivity between circadian rhythms, sleep and immune function, and their implications for public health. A key concept worth considering is that, both circadian rhythm and sleep disruption happen concurrently every day, and this is especially true in modern life through the widespread experiences of shift work, light at night, and social jet lag[53].

2 Person - Chronotype

Timekeeping is not universally the same in populations, and we all possess the potential for different 'settings' of the clock. These versions – or chronotypes - fall on a continuum, at the either end of which are individuals colloquially referred to

as morning larks and night owls. Larks have a morning type ('morningness') and prefer to awaken early in the morning and feel most active during the earlier parts of the day, while owls have an evening type ('eveningness'), preferring to awaken later in the day and typically feel most active and motivated in the late evening or at night. Individuals who fall between the ends of the continuum are referred to as intermediate types[54]. While chronotype is largely imprinted in small differences in our clock genes, changes in morning/evening predisposition adapts with life span with morning preference during childhood development, evening type in adolescence and early adulthood, then progressively morning preference with age advancement[55,56]. Chronotype is a risk factor for cardiometabolic and mental health, with evening types being more susceptible to conditions such as obesity and bipolar disease[57,58]. A systematic review of evidence has also shown a trend for evening chronotypes to experience more serious symptoms for immune-mediated inflammatory diseases[59]. There is an appreciation that sex as a biological variable might impact immune responses. For example, adult females it seems produce higher antibody responses to diverse vaccines against, influenza, hepatitis B, yellow fever, rabies, herpes, and smallpox viruses[60,61]. However, females may experience greater vulnerability to circadian misalignment from factors like shift work, possibly because females are more likely to be classified as morning types as compared to males meaning that there is potentially more severe misalignment with the 24-hour day-night cycle imposed by shift work[62].

3 Place - Circadian clocks and our modern lifestyles

Modern life and our environment also shape chronotype. Roenneberg & Merrow[18] describe two distinct environmental scenarios influencing clock entrainment; modern/industrial and ancestral/rural entrainment of the clock by weak and strong zeitgebers, respectively. Modern/industrial entrainment features indoor light and artificial light at night and a concomitant reduction in the difference between the maximum and minimum amount of light exposure during the day and the level of darkness at night. This environment predisposes individuals to later chronotypes because of the blurring of the alignment of internal clock rhythms with 24-hour day-night patterns. This contrasts with ancestral/rural entrainment featuring bright daylight and no artificial light at night, where clock rhythms are effectively synchronised to the 24-hour day-night pattern. Seasons also modulate chronotype; in winter, chronotype is also later, probably due to a combination of later sunrise and lower light levels[63,64]. Therefore, a wide range of factors inherent in modern life and our environment influence chronotype – although genetics and age play roles, external factors such as light, social activities and school or work schedules can mask our endogenous chronotype.

Light at night is a hallmark for our modern lifestyle and is thought to be a key driver of social jetlag – a condition resembling a mild but chronic form of shift work and epidemiological studies show that this correlates with health problems, including the tendency towards being overweight. Modern life can therefore be thought of as living against the circadian clock in that it induces a sub-optimal form of entrainment, a mis-match between the natural rhythm of our light:dark environment and our physiology driven by our internal, or endogenous, circadian clock[18]. The evening, or 'owl' chronotype – typical of teenage years – appears to be particularly at odds with our modern society that is skewed towards morning-orientated activities such that individuals are forced to live in a state of circadian misalignment, the schism being their innate tendency to wake late and the societal demands of attending school or work in the morning[65]. A study of chronotypes in school age children (8-12 years) showed that evening types have altered sleep patterns, social jet lag, higher BMI and higher metabolic risk (higher values of insulin, glucose, triglycerides and cholesterol)[66]. Also, while light at night not only shifts populations towards later chronotypes, modelling studies suggest that it also has the tendency to reinforce evening preference and exacerbate circadian and sleep-related problems associated with the owl chronotype[67].

In 2022, around 27% of the UK workforce were employed in the evening or during the night[1], compared to a reported 16% of adults who usually work a non-daytime schedule in 2018 in the US[17]. Working indoors, in low-light or artificial light conditions with relatively little blue light, does not provide the same light exposure as natural daylight, and is a risk factor for disruption to the harmonious relationship between our endogenous clock rhythms and the external environment. Natural daylight at high intensities has been shown to be particularly beneficial for advancing the timing of sleep to earlier hours thereby lengthening the duration of sleep, and for improving sleep quality[68]. A 2002 publication estimated that around 70% of US workers are employed in indoor work environments[69].

Outside our homes and work environments, the biological effects of light pollution on human wellbeing and natural ecosystems are significant, exemplified by a UK Royal Commission on Artificial Light in the Environment[2] that recognises the explosive growth in outdoor lighting in the UK since the 1940's – and the growing sense of loss of 'dark skies' together with the resultant cultural disconnect[70]. The Royal Commission comparison of light at night light from 1993 to 2000 shows that almost every area in the UK has become brighter, particularly rural areas such that in 2003 only 22% of England possessed pristine night skies compared to 77% for Scotland, although in Scotland the main populated areas stretching from Glasgow to Edinburgh – the 'central belt' - shows almost unbroken levels of light pollution, creeping out from the cities and towns to blur any distinction between urban and rural areas[3]. The Royal Commission also recognised that since light is perceived as a natural and benign phenomenon, the insidious and negative issues of 'light blight' on our natural world are overlooked when any other anthropogenic impact would naturally provoke stronger interogation, although artificial light as a neglected pollutant was recognised as a public health issue in a 2023 UK House of Lords Science and Technology Committee investigation[4].

Place – rurality or urbanity for example – influences the daily synchronisation of our internal endogenous clock with the light:dark environment, conceptually emboldening a harmonised, ancient, form of entrainment or a mis-matched, modern, socially jetlagged, form of entrainment, respectively. Although light is thought of as the principal zeitgeber, clock epidemiologists have wondered whether social cues – hardwired into the fabric of our lives by social constructs such as school or work times and governed by digital clock time – can counter natural light:dark cues; after all rurality does not preclude a modern lifestyle. Roenneberg *et al.*[13] teased apart this question of the relative balance of the coupling of the circadian clock to natural 'sun time' or to constructed 'social time' in a population sized study – disentangling the cues using the principle that within a Time Zone people and live and work to a common social time – constant over multiple longitudes – that is at odds with sun time that continually changes across longitude. The population study used individuals in towns and cities across Germany and measured their responses to a questionnaire based around their estimated chronotypes, finding that although the clock was principally entrained by sun time, individuals in lightly populated areas were more tightly

[1] Office for National Statistics (ONS) The Night-Time Economy, UK: 2022 https://www.ons.gov.uk/businessindustryandtrade/business/activitysizeandlocation/articles/thenighttimeeconomyuk/2022

[2] The Royal Commision on Environmental Pollution. Artificial Light in the Environment (2009) https://www.gov.uk/government/publications/artificial-light-in-the-environment

[3] Campaign to Protect Rural England. Night Blight: Mapping England's light pollution and dark skies (2016) https://www.cpre.org.uk/resources/night-blight-2016-mapping-england-s-light-pollution-and-dark-skies/

[4] House of Lords Science and Technology Committee 2nd Report of Session 2022–23. The neglected pollutants: the effects of artificial light and noise on human health (2023) https://committees.parliament.uk/publications/40937/documents/199438/default/

coupled to sun time than those living in densely populated areas, leading the authors to conclude that the uncoupling was a reflection of the emboldening of social cues versus nature's light–dark cycles. Tipping this balance from environmental towards behavioural light–dark cycles is predicted to lead to later chronotypes, a characteristic increased population size[13]. Our preference for aligning our clocks to sun time rather than clock time is seen with the effects of travel across Time Zones, where the clock dynamically re-entrains to external light/dark cycles and environmental cues in their new environment, with faster recovery from jetlag after westward, as opposed to eastward, travel, possibly as a result of a preference for the clock to delay, rather than advance, its entrainment[71]. Where we reside in a Time Zone contributes to our harmony with sun time – living on the western edge of a Time Zone where light later in the morning extends to later in the evening because of delayed solar time can result in circadian misalignment resulting in significant deleterious effects on human health outcomes[72,73]. For example, cancer-specific mortality has shown to be a function of the distance from the eastern border of a Time Zone, with risks increasing and longevity decreasing from east to west[74]. Reductions in sleep duration due to extra natural light at night because of the 'western edge effect' was also found to have significant adverse effects on human health and on economic performance (per capita income)[75]. Spain, geographically aligned with Greenwich Mean Time (GMT), operates on Central European Time (CET), while neighbouring Portugal operates on GMT. This discrepancy between social time and sun time leads to later bedtimes and meal schedules in regions northwestern Spain, potentially affecting sleep and overall circadian rhythmicity[73].

4 Time – Immune function

Despite adaptive immunity occurring over a long period of time compared to the innate 'first line of defence' response – weeks compared to days[76] – studies have suggested that the timing of the initial immune stimulus can have lasting effects on immune (patho)physiology[76–79]. This has led to the question as to whether daily immune rhythms could be used to target the adaptive immune system at the time of highest sensitivity to bring about enhanced vaccination responses[80]. Experimental evidence for variation in antibody production after vaccination has been obtained[81–85], for example a large, randomised trial of different times of vaccination showed that morning vaccination enhanced the antibody response to the influenza vaccine[84]. There has been some scepticism about the role of the clock in the development of adaptive immunity[86] – after all, how can a 24-h rhythm influence adaptive immunity responses forming over days and weeks? However, the step-by-step generation of the adaptive immune response over several weeks has been traced, where it was found that the timing of the initial immune challenge was imprinted on all subsequent downstream processes[76]. Taken together these studies suggest a 'sweet spot' at or just before the behavioural activity phase for optimal vaccination – correlating with morning vaccination timing for human subjects.

While daily timing is the focus of TODV, it seems that immune function is also sensitive to another predictable timing dimension – seasonality – the result of the axial tilt of the Earth combined with its orbital path around the Sun. A large population study of UK Biobank participants not only showed daytime variation in immune parameters, but also a seasonal variation, with lymphocyte numbers showing the greatest contrast[64]. Daylength modulates with season and both the intensity and spectral composition of light to which people are exposed vary with season[87], and while direct evidence for seasonality in humans is limited[88], in animals the seasonal clock is generated by changes in thyroid hormones in the brain that respond to day length as signalled by the pineal hormone melatonin. A study collecting light quality across seasons using wearable colour-sensitive 'actiwatch' devices in human subjects showed that summer subjects were exposed to twice as much blue light than winter subjects[87]. The spectral sensitivity of the photopigment melanopsin, located in the

human inner retina is known to be greatest for blue light and has been shown to mediate many aspects of non-vision related human behaviour and physiology such as sleep timing and daily melatonin rhythms[87]. The relative contribution of blue light is therefore greater in summer compared to winter, and while blue-enriched light has positive effects on sleep quality, alertness and performance[89], the timing of blue light is important such that exposure at night with the overuse of electronic devices rich in LED exacerbates social jetlag[90,91], the resultant disrupted clock affecting the timing of hormone secretion, the activity of immune cells, and body temperature, and changes in mood at different times of day and night[92], and has been shown to be a risk factor for psychological disorders[93], and obesity[94].

In comparison to the well characterised circadian clocks, other biological timers – circatidal clocks, linked to the ~12.4-h tidal cycle, and circalunar clocks, cycling every ~29.5 days – are less well understood and hotly debated to the present day[95]. Both circalunar rhythms and circatidal rhythms are widespread in marine environments, but recent evidence suggests their potential influence on human health[96]. Experiments in sleep laboratories, in which external light sources such as moonlight were systematically excluded, show the effect of the lunar cycle on sleep, with deep sleep patterns and total sleep time negatively and significantly reduced around full moon[97]. Circatidal clocks are used by marine organisms to anticipate changes linked with the ebb and flow of tides, such as water levels, food availability, currents and temperature[95] and a recent seminal study in humans intriguingly demonstrated 12-h rhythms in gene transcript levels[98]. However there remains a debate as to the potential function of a 12-h mammalian clock - after all why would terrestrial animals need a ~12 h rhythm generator if they are not subjected to tides. One line of thinking is that a mammalian 12-h clock is either an evolutionary remnant of the marine circatidal clock or has evolved convergently, and that it facilitates the 'rush hour' processing of elevated gene expression and processing around dawn and dusk[99]. The clear characterisation of circatidal and circalunar rhythmicity in humans is challenging at the practical level, requiring strict control of light sources, as well as meal and sleep timing, and there remains the problem of elucidating if 12-h rhythms are mechanistically driven by the circadian clock, rather than by a dedicated oscillator, or simply driven by environmental cues such as light[95]. A link between immune function and the potential twice daily circatidal rhythm anticipation of metabolic stress or circalunar rhythm sleep pattern modulation has not been made. Immunity, sleep, and metabolism are however intricately intertwined and understanding the influence of ultradian rhythms (period shorter than a day) and infradian rhythms (period longer than a day) on immune function homeostasis will pave the way to appreciating immune processes across different timescales.

5 Person, Place & Time

Populations therefore feature a rich mixture of chronotypes, young/old clocks with natural variation in our genetically predetermined lark and owl propensities interacting with, and ultimately being shaped by, their surroundings; for example, rural/urban settings and summer/winter light environments. Generally, less is known about how secondary oscillators, in peripheral organs (brain, heart, liver, gut, pancreas, adipose tissue, adrenal glands, lungs, and skeletal muscle[12]) entrain with the SCN central pacemaker and how potential misalignment mechanistically contributes to human health. Roenneberg & Merrow[18] point out, however, that there is likely to be a constellation of possible phase entrainments, or 'phase maps', describing the entrainment between the central and peripheral clocks; reflecting the idea that we entrain differently at different times of our lives, at different times of the year and amongst each other, and whether we live in cities or in the country[18]. Table 1 summarises Person, Place & Time variables relevant to chronovaccination studies.

Table 1: Person, Place & Time variables for chronovaccination studies

6 Chronovaccination

Naturally, the question as to whether daily variations in our immune response extends to our antibody response to vaccination has been posed. Early work showed mixed evidence for effects of time of vaccination on antibody response, possibly as a reflection of low sample sizes, variable design, short-term follow-up, and differences in vaccine types. However, a large-scale cluster-randomised trial examined the influence of the timing of vaccination on influenza antibody titres and found that morning vaccination enhanced the antibody response to the influenza vaccine[84]. Using a prospective cohort study design Zhang *et al.*[100] showed that vaccination in the morning lead to a stronger immune response to SARS-CoV-2 vaccine. In their 2022 review, Otasowie *et al.*[1] coined the term 'chronovaccination' – a term encapsulating the evidence of clock gating of the immune response and the potential of TODV for maximising vaccine immunogenicity. The authors highlighted four clinical studies demonstrating TODV, including the aforementioned first large-scale randomised controlled trial assessing the effect of different vaccination times for the influenza vaccine[84], an assessment of the effect of TODV on specific and non-specific immunity induced by the TB vaccine, Bacillus Calmette-Guérin (BCG)[81], and for two prospective cohort studies, including the aforementioned Zhang *et al.* study, assessing antibody responses after administration of SARS-CoV-2 vaccines at different phases of the day[100,101].

The Otasowie *et al.*[1] review, however, sets out conflicting evidence for the optimum TODV. The work by Zhang *et al.*[100] demonstrated that vaccination in the morning (9 am - 11 am) compared to vaccination in the afternoon (3 pm - 5 pm) lead to a stronger immune response to an inactivated SARS-CoV-2 vaccine (BBIBP-CorV, Sinopharm), while in contrast Wang *et al.*[101] showed, with vaccinating mRNA or Adenovirus based SARS-CoV-2 vaccines (Pfizer, mRNA bnt162b2 or AstraZeneca, Adenoviral AZD1222) in a larger cohort, that antibody responses were higher in those who were vaccinated later in the day (11 am - 10 pm) compared to those vaccinated in the morning (7 am - 11 am). Wang *et al.*[101] speculated that the use of the inactivated whole virus in the Zhang *et al.*[100] study might explain the difference in the TODV outcomes, and note several other potential factors complicating interpretation of the outcomes including altered sleep and shift-work patterns of the healthcare worker participants of the study potentially influencing vaccine responses.

Recently Hazan *et al.*[102] carried out a retrospective large population cohort analysis of timestamped COVID-19 vaccinations (1.5M individuals, age $>$ 12 yo, 99.2% receiving Pfizer-produced BNT162b2), using COVID-19 breakthrough infection and COVID-19–associated emergency department visits and hospitalisations as endpoints for cohorts split by vaccination timing (morning, afternoon, or evening). This study found lowest rates of breakthrough infection with late morning to early afternoon vaccination and highest rates associated with evening vaccination. Vaccination timing remained significant after adjustment for patient age, sex, and comorbidities. The benefits of daytime vaccination were concentrated in younger ($<$20 years old) and older patients ($>$50 years old)[102]. Although vaccinations were not performed across 24-h cycles, the authors were able to demonstrate that the risk of COVID-19 breakthrough infection as a function of vaccination timing was sinusoidal in shape, displaying a periodicity of between 7.4 and 16.7 hours, considerably shorter than the classical 24-h periodicity of circadian rhythms, suggesting that rhythms in vaccine effectiveness are potentially ultradian in nature, for example driven by a mammalian 12-h clock[102]. Another retrospective large population cohort study (0.25M individuals, age $<$ 6 yo), this time for the varicella vaccine, showed that vaccination in the morning and

afternoon was associated with lower infection rates than evening vaccination[103]. Like that for the risk of COVID-19 breakthrough infection, varicella infection risk, after adjusting for ethnicity, sex, immunodeficiency, and obesity, followed a sinusoidal pattern consistent with a diurnal rhythm in vaccine effectiveness. Exploratory post-hoc analysis of a clinical trial (DANFLU-1[104]) of the relative effectiveness of high dose versus standard dose quadrivalent influenza vaccines established that earlier TODV was associated with fewer respiratory hospitalisations[105], although the authors noted that this association was likely limited by relatively few outcomes in subjects vaccinated later in the day.

Two independent systematic reviews[2,106] have surveyed published studies on the human immune response to vaccination at different times of day in order to gauge the evidence supporting diurnal variation in the effectiveness of vaccination. Wyse *et al.*[2] noted the challenge in generalising the findings based on the heterogeneity of the studies surveyed, however the authors concluded that overall there was insufficient overall evidence that administration of vaccines at different times of day affected immune outcomes. On the other hand, Vink *et al.*[106] concluded that that morning vaccination enhanced antibody responses in adults aged 60 years and older, noting that this age group is a key demographic for influenza and COVID-19 vaccination. Studies qualified for inclusion in the Vink *et al.* review if they measured antigen-specific antibody or T-cell responses following vaccination, and if these immune responses were compared between participants vaccinated at different time points during the day, whereas for Wyse *et al.* studies were included if they reported any immune or clinical outcome following vaccination at a defined time of day.

The Wyse *et al.*[2] review assessed over 3,100 studies, 23 of which met their inclusion criteria. These studies were published between 1967 and 2024 and almost half of them included reported data collected for SARS-CoV-2 vaccination programs during the COVID-19 pandemic. The review authors found that most studies were biased by failing to account for immune status prior to vaccination, self-selection of vaccination time, or due to the presence of confounding factors such as sleep, chronotype, and shift work. Of these 23 studies, the optimum TODV was concluded to be afternoon (5 studies), morning (5 studies), morning and afternoon (1 study), midday (1 study), and morning or late afternoon (1 study), with the remaining 10 studies reporting no effect. Of the studies that reported an association between TODV and outcome of vaccination, 3 presented data that could be used to estimate the size of this effect[100,102,107]. For example, Hazan *et al.*[102] estimated that optimising the time of vaccination might improve vaccine effectiveness by 8.6–25%.

Vink *et al.*[106] identified 860 records through their literature search, 17 of which met their eligibility criteria; five investigated the effect of vaccination timing on immune responses to influenza vaccines[84,85,108–110] and nine explored how time of day influences immune responses to SARS-CoV-2 vaccines[100,101,107,111–116]. Eleven out of the 17 studies reported statistically significant effects of vaccination timing, with ten reporting stronger antibody responses following morning vaccination, while one study favoured vaccination later in the day. The strongest evidence for diurnal variation was found for influenza vaccines in older adults; sub-group analysis for age stratification in the included studies consistently showed stronger antibody responses for 60-year-olds or older for morning vs afternoon vaccination. Pooled results from two randomised controlled trials[84,108] for influenza vaccination showed a statistically significant small-to-medium standardised mean difference (SMD) in antibody titers for adults aged 65 and older (effect size; SMD = 0.32, 95% CI = 0.21–0.43), with morning vaccination consistently yielding higher titers 1 month post-vaccination. However, Vink *et al.*[106] caution over-interpretation of this reported effect size due to the limited number of randomised controlled trials used in the meta-analysis.

7 Discussion

The role of circadian rhythms in many diverse strands of modern society are increasingly apparent, in areas such as health and well-being - from increasing understanding of chronic clock disruption (e.g., shift work, social jet lag) through to optimal living – the integration of personal circadian rhythms into daily schedules for eating, exercising, and sleeping to maximise health and performance, increasingly regarded as a 'game changer' in elite sports regimes[24]. Another example, in agriculture – harnessing knowledge of crop clocks to increase yields and resilience, more and more topical with the gathering storm of climate change and food security challenges[28]. Both examples under the cloak of cultural disconnect from natural cycles of light and dark and the insidious creep of artificial light, including blue light at inappropriate phases of our day. Modern lifestyles embolden living against our natural clock settings, yet decades of fundamental scientific research in biological clockwork, rewarded with the 2017 Nobel prize in Physiology or Medicine[5], are offering opportunities to harness aspects of clock function as chronotherapies. One such therapy – chronovaccination - might offer an extra edge in vaccine effectiveness. However, is the current evidence strong enough to support the idea that it can be a low-cost, low-risk strategy for boosting vaccine effectiveness? The case for chronovaccination – surveyed in systematic reviews[2,106] – is somewhat patchy. While the influence of TODV is compelling in animal studies where environmental conditions are strictly controlled[76,80], human populations feature a panoply of individual clock types set in a multitude of environments. Young clocks, teenage clocks, old clocks, with their spectrum of lark and owl clock 'settings'. Clocks in rural and modern industrial environments, seasonal clocks, socially jetlagged clocks - all muddling interpretation of human circadian studies assessing the effect of TODV. Large population cohort epidemiological studies have been successful in teasing apart relationships between our endogenous clock function and the environment – for example revealing the association between where we live in a Time Zone and chronodisruption[13]. Population-level approaches have also been used in chronovacination studies[102,103], but report modest effect sizes and suffer from potential biases due to non-randomisation of cohorts. Vink *et al.*[106] demonstrated that the strongest evidence for diurnal variation in vaccination effectiveness was found for influenza vaccines using randomised control trials and Wyse *et al.*[2] argue that large population randomised trials are the ideal, with the power of thousands of participants enabling adjustment for multiple demographic and lifestyle factors that might otherwise confound detection of an effect of TODV. These would be costly enterprises, but Wyse *et al.*[2] reason that the onus of determining whether effectiveness is a function of the TODV should fall to the vaccine producers during clinical trials.

Even if there was a compelling advantage of TODV, would it be readily integrated into vaccination programmes? One only has to look at the debate around Daylight Saving Time (DST)[117] to understand the sensitive nature of policies that have disruptive effects on social routines. Another relevant example is the idea of later school start times for adolescents – designed to align better with their clock driven 'owl' behaviours – that illustrates how interventions, however well intended, can shift the problem onto other groups of individuals, in this case later scheduling working against teachers and parents own chronotypes and social schedules. Would the potential benefit of TODV justify its disruption to well-established vaccination programming? On the one hand boosted vaccine effectiveness can help improve vaccine hesitancy, with hesitancy identified by the WHO as one of the 10 threats to global health[6]. On the other hand, the convenience of accessing vaccinations is an

[5] Nobel Foundation, The Nobel Prize in Physiology or Medicine 2017 - Press Release https://www.nobelprize.org/prizes/medicine/2017/summary/

[6] Understanding the Behavioural and Social Drivers of Vaccine Uptake, World Health Organization (2022) https://www.who.int/publications/i/item/who-wer9720-209-224

important factor for vaccine uptake[2], and programs scheduled across much of the day offer individual choice and convenience that may be expected in a 24-7 society. Conceivably immunisation initiatives more narrowly aligned to an optimum TODV may be detrimental to vaccine uptake, although nudging based interventions have shown potential in bolstering intention to vaccinate[118], and may therefore be a useful strategy for fostering confidence in time-of-day vaccinations.

While the benefits of TODV across whole populations remains marginal, a clearer case can be made for chronovaccination in adults 60 years and older, where morning vaccination was more beneficial[106]. It has been speculated that immunosenescence - the gradual age-related decline in both innate and adaptive immune function - may play a role[108,119]. Similarly, immunosuppressed individuals might benefit from TODV-mediated boosted vaccine effectiveness[115,116], suggesting that TODV may find a role in targeted age group and co-morbidity cohorts. The potential of infant chronovaccination is largely an unexplored area, with most studies focussed on adult COVID-19 and influenza programmes. A recent study, however, examined diurnal patterns of varicella vaccine effectiveness in children - finding that immunisation during the late morning to early afternoon led to fewer breakthrough infections compared with those vaccinated in the evening[103]. And what of urban-rurality? How might 'Place' - the extent of coupling to 'sun time' - influence sensitivity to TODV? This and the influence of seasonality, for example, on TODV are largely unexplored and might contribute to understanding the complex relationship between the environment, clocks, and immune function. Although a discussion of the role of sleep in immune function, and therefore its contribution chronovaccination, is not developed in this review, Livieratos *et al.*[120] have reviewed the evidence of the impact of circadian and sleep factors on influenza vaccine-induced immune responses, emphasising that yet another variable – sleep duration – should be considered for understanding vaccine-induced antibody responses.

It was hoped that the award of the clock biology Nobel Prize in 2017 would emphasise the time-of-day reporting in biology and medicine. However, Nelson *et al.*[37] report that the time-of-day variable remains largely ignored. Much commentary on the future direction of chronovaccination emphasise the importance of systematically recording the time-of-day at which vaccines are administered, yet to many chronobiologists it remains a puzzle why such a fundamental biological variable is regularly overlooked in the first place. One factor may be the increased resources and costs that accompany properly designed multi time-point circadian assays; however circadian biologists would no doubt argue that biological studies cannot be reliably replicated – a fundamental tenet of science – unless time-of-day is recorded. Another important consideration is that internal biological time (circadian phase) can vary considerably, the consequence of modern lifestyles and the high chance of desynchrony due to many of the Person, Place and Time factors described here (Table 1). For example, nurses' circadian rhythms in body temperature have been shown to be significantly misaligned due to shift working patterns, causing work and sleep schedules to be out of phase with each other[121]. Both external time of vaccination and internal (biological) time recording are therefore important factors, with internal time recording representing a particular technical challenge[53]. However, research on circadian wearable devices that continuously track body movement, heart rate, temperature, and light exposure has increased significantly over the past decade, are continually improving and offer a non-invasive method to capturing real-life circadian rhythms[26].

'Time will tell' if chronovaccination finds a role in delivering an edge to vaccine effectiveness. There remain many challenges, not only in establishing clear clinical benefits, but other hurdles akin to practical and societal issues. The general public's growing interest of the role of circadian rhythms in relation to health and well-being, or 'circadian hygiene', might ultimately drive individual awareness of optimal times for vaccination. Therefore, as Vink *et al.*[106] allude to in their review, the future

vaccine customer may well regard optimal vaccination as an hourly continuous variable in relation to their personal circadian phase rather than the more blunt binary (morning *vs* afternoon) variable we currently consider. In this regard it will be particularly interesting to learn the outcome of the 'Chrono-Vax' clinical trial that aims to determine the effect of influenza vaccination timing on antibody and T-cell responses across a continuous time window from early morning to late afternoon for participants aged between 60 and 85 years old[122]. Importantly, participants will be asked to provide data on their history of shift work, comorbidities, (sleep) medication use, lifestyle characteristics, as well as insights into their chronotype[122].

8 Conclusions

Chronovaccination studies have been demonstrably hampered by the panoply of confounding factors inherent to population structures and our modern lifestyles. Understanding endogenous human biological time-keeping in the context of their exogenous settings is likely key to disentangling the relative influences of covariates. Nonetheless, TODV as a chronotherapy shows promise for older age and immunosuppressed groups, and while the effect size may prove to be modest, their practical significance could be viewed as a marginal gain in vaccine effectiveness, in the same way that knowledge of clockwork is harnessed as a competitive advantage in numerous other areas of society. Key hurdles in the adoption of chronovaccination are whether society buys into the idea and whether health agencies conclude that the fragility of vaccine uptake might ultimately be further jeopardised by changes to well established vaccination programmes. While the timing of vaccinations may not be at the forefront of our minds during interpandemic peace times, continued research on the potential benefits of chronovaccination should be an important strand of our approach to preparing for future pandemics.

9 Acknowlegements

I recognise that the scope of this review, due to space restrictions, prevents the citation of every relevant publication. I apologise for any omissions of important work in this topic area.

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