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How Blood Could Age the Brain

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Abstract

Our memories deteriorate across the lifespan, and this poses increasing public health challenges. A brain structure known as the "hippocampus" is essential for establishing memories and, critically, may also be the only site where new brain cells (neurons) are produced throughout adulthood (adult neurogenesis). Neurogenesis appears to be important for normal memory function, but it decays with age, while conversely, exercise increases it. Though the causes remain unknown, it has been found that proteins and metabolites in the blood could regulate adult neurogenesis, and that the levels of these molecules change as we age and with physical activity. In my research, I aim to identify proteins and metabolites that display opposing relationships with ageing and exercise, which could provide early detection for cognitive decline and new targets for intervention. I recently identified a protein (known to occur in new brain cells) in the hippocampus and three metabolites in blood that are responsive to exercise and ageing, with one metabolite displaying an opposing relationship. By comparing these results with future analyses of proteins in blood and cerebrospinal fluid (which envelopes the brain), I hope to arrive at a mechanistic pathway by which exercise could ameliorate age-related cognitive decline via neurogenesis.

Keywords: neurogenesis, ageing, exercise, memory.

There is no biological law that says we must age.

— David Sinclair

Age-related cognitive decline - a formidable challenge

By the year 2050, the global population aged 60 and over is predicted to have doubled. This means an ageing of society's collective cognitive capacities. The consequent rise in dementia and societal costs will pose formidable challenges to public health. Among the mental faculties to decline with age, episodic memory (i.e. memory for everyday events) is a particularly poignant case, as its deterioration entails not only impediments to everyday activities but – perhaps more significantly – a fading of life's chronicle.

Central to episodic memory is the integrity of a brain structure known as the "*hippocampus*". This seahorse-shaped region performs the unique feat of establishing a record of the brain's entire activity state in any given moment, and critically, can trigger a near verbatim replay of that state, thus allowing for the mental time travel that we associate with reminiscing. While the sights, sounds, smells, and feelings of an event are generated across the entire brain, it is the hippocampus that acts as a conductor – particularly during sleep – until the memory trace is firmly laid down in the connections between brain cells, rendering the memory permanent. Without the hippocampus, there would be no new memories – no "*what-happened-where's*" – and I would not be able to recall that June 2019 Billie Eilish concert in Stockholm with such brilliant vividity.

Life invariably poses another challenge to the brain: the need to remember highly similar and related events as distinct. For instance, one's many birthdays are often spent with the same people in similar locations; you frequent the same shops and restaurants; your workdays follow predictable schemas, and yet, these events do not simply overwrite each other. This problem is solved through a mnemonic splitting of hairs called "*pattern separation*", which takes place in a sub-region of the hippocampus known as the "*dentate gyrus*". The neurons (brain cells) of the dentate gyrus are able to convert small differences in input (e.g. wearing your blue jacket to work today as opposed to the red jacket you wore yesterday) into large differences in output (i.e. the degree of overlap of memories). If pattern separation falters, the overlap of similar memories increases, and the rest of the hippocampus cannot disambiguate them, causing memory interference. Notably, pattern separation – compared to other forms of memory – is particularly susceptible to age-related decline. While the cause remains unknown, it may be related to another unique phenomenon harboured within the dentate gyrus: the ongoing production of new neurons throughout life (*adult neurogenesis*).

Adult-born brain cells - critical for memory

In 1965, an observation was reported that would call into question a central dogma of 20th century neuroscience: within the adult nervous system "*nothing may be regenerated*". It was widely held at the time that all neurons are generated before and shortly after birth and that no cells divide to give rise to new neural progeny during adulthood. Two scientists at the Massachusetts Institute of Technology, Joseph Altman and Gopal Das, tested this claim by injecting a *nucleotide* (a building block of DNA) labelled with radioactive hydrogen into adult rats. Critically, the nucleotide is only incorporated into cells that are dividing (during which DNA synthesis occurs) and, hence, in recently produced cells. When examining the rat hippocampus, Altman and Das discovered what appeared to be radioactively labelled neurons in the dentate gyrus, providing seminal evidence of ongoing adult neurogenesis. Though initially met with scepticism, the field of adult hippocampal neurogenesis garnered significant attention in 1998 when Peter Eriksson and colleagues found evidence of adult-born neurons in human cancer patients who had undergone diagnostic treatment with another nucleotide to

track tumour development. The phenomenon has since received experimental support from many studies employing different methodologies.

Currently, there is ongoing debate as to the extent of human adult hippocampal neurogenesis, with some notable studies failing to find evidence of it, while others suggest the process is ongoing well into the 10th decade of life. Yet, one trend has emerged from both human and animal studies: neurogenesis is not constant throughout life; rather, it steadily declines as we age. This decay could drag our memories down with it, as studies in rodents have revealed neurogenesis to be important for ensuring the distinct storage of similar memories through pattern separation. Yet, why should neurogenesis dwindle with age at all, and is it *truly* inevitable?

Blood ageing begets brain ageing

The cells responsible for the adult-born neurons are located in a highly specialized microenvironment in the dentate gyrus that is comprised of other brain cells and signalling molecules (e.g. proteins – the machines of life – and metabolites – chemical breakdown products), which can regulate neurogenesis. The environment is also highly vascularized, possibly allowing blood-borne factors to impinge upon the process. This is important, as the contents of the circulatory system are a reflection of the state of the entire organism. For instance, when we are sick, immune cells release molecules (*cytokines*) that signal the presence of infection. When we exercise, muscles secrete metabolites and hormones that carry salutary effects to the rest of the body. However, blood content also varies over the lifespan. A steady rise in inflammatory cytokines has been observed with increasing age (an effect now known by the portmanteau "*inflamm-aging*"), and the level of these molecules in middle-aged people are predictive of cognitive decline 20 years later. In rodents, the hippocampus has been found to suffer the same inflammatory fate, and notably, this is accompanied by the conspicuous waning of neurogenesis. This begs the question: are the two phenomena causally linked?

The idea was put to the test using a 19th-century method called "*parabiosis*", wherein the circulatory systems of two animals are surgically joined. In 2011, a research team at University of California created such conjoined pairs with mice of different ages. Notably, when young and old mice shared blood, the levels of hippocampal neurogenesis declined in the young animals, but they more than doubled in the old (as compared to same-aged duos). Young blood, thus, seemed to rejuvenate the ageing hippocampus, while old blood aged it. The culprits were found to be inflammatory cytokines. Not only did their levels increase with age in the circulation of animals, but the same trend was confirmed in human blood and cerebrospinal fluid (the liquid that envelopes the brain and spinal cord). More importantly, the impact of aged blood on neurogenesis was accompanied by memory impairments. This raises the possibility that brain ageing could be ameliorated by delaying blood ageing. Among the most studied interventions to this end is a surprisingly simple one: physical exercise.

Running to rejuvenate the brain

In the early 2000s, it was discovered that mice housed with running wheels had higher levels of adult hippocampal neurogenesis than mice housed without. Running seemed to cause cells to divide more frequently. Intriguingly, it also appeared to improve the distinguishing function of the dentate gyrus – pattern separation – for which the adult-born neurons were thought to be important. The latter results were soon extended to humans: improving one's maximal aerobic capacity also improved one's ability to tell highly similar memories apart. Yet, how is this body-to-brain communication achieved? Physical exercise has long been appreciated for its anti-inflammatory benefits, and skeletal muscles are known to release molecules into the bloodstream. Could the opposing effects of ageing and exercise on neurogenesis and memory be the result of opposing blood profiles?

A Stanford University research team led by Tony Wyss-Coray tested this idea by transferring blood from young mice that were housed with or without running wheels to aged sedentary mice. The mere administering of blood from exercised mice was nearly as effective as running itself on the birth of new neurons in old mice. Once again, circulating proteins were responsible for the body-to-brain translation, and one of them (an enzyme that breaks down fatty acids) was similarly found to be elevated in elderly humans who walked at least 7100 steps a day. In a follow-up study with only young mice, the runner's blood treatment both increased neurogenesis and lowered inflammation in the entire hippocampus; the culprit was a blood clotting protein whose levels were also responsive to exercise in humans. Perhaps then, to rejuvenate the brain, we could rejuvenate the *blood*, and we might do it by *running*.

The current project: exercise to cure middle age memory

To summarize the decades of research above: the *dentate gyrus* generates new neurons throughout life (*adult neurogenesis*), and this process is important for memory (*pattern separation*); blood is a reflection of the state of the organism and can impact neurogenesis – and consequently *memory* – for better (with *exercise*) or worse (with *ageing*). Thus far, the focus has been on blood alone, but if we could profile the changing contents of the circulation and brain together, we might unravel both the causal molecules and the mechanisms whereby they affect neurogenesis and memory. This could yield new druggable targets and tractable diagnostic markers of early cognitive decline. Such an endeavour should also involve cerebrospinal fluid, which serves as a reflection of the state of the brain and interfaces with blood. For instance, its contents predicts the development of myriad neurological diseases, such as Alzheimer's, often years – or even decades – before symptoms begin to show. Much research on ageing involves the extreme end of the lifespan, when effects are likely to be large and easy to observe, but by then, it may be too late for any intervention to undo the attrition of time. Therefore, we should study a period of life that is both malleable and prognostic. *Middle age* may be such a period: at this stage, blood-borne inflammatory cytokines become predictive of cognitive decline two

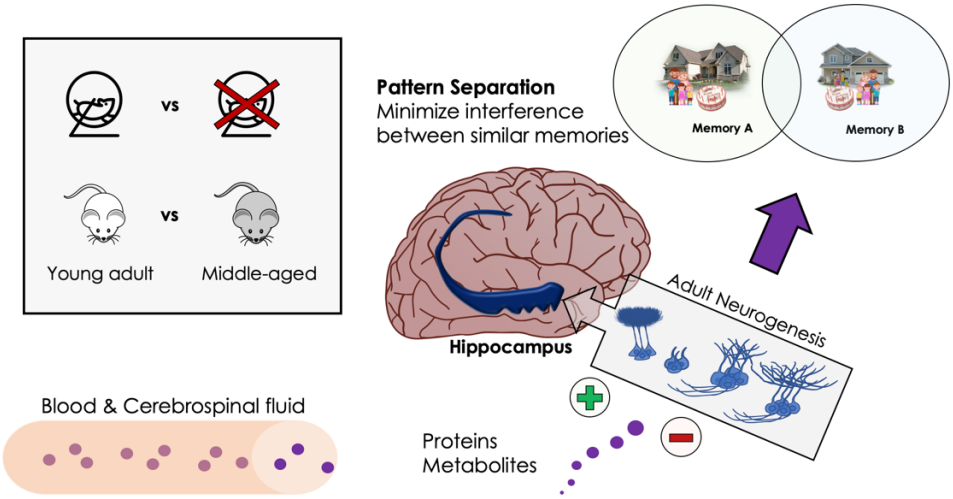

Figure 1: Project overview. Ageing and excerise alter protein and metabolite levels in blood and cerebrospinal fluid, which can regulate adult hippocampal neurogenesis and, consequently, pattern separation.

decades down the line; inflammation becomes apparent in the rodent brain, and the relationship between dentate gyrus volume and pattern separation ability in humans displays a turning point. Could neurogenesis be the unifying motif? Which are the circulating culprits that help or hinder it? Could exercise turn back our biological clocks to reverse the effects of ageing?

I hope to shed some light on these questions during my PhD. What is uniquely powerful about our project is that we will be employing both *animal models* (in which brain tissue is easier to come by and biological mechanisms are more readily investigated) and *human participants* (whom we hope to benefit in the end but are ethically challenging to study). By comparing results from blood, cerebrospinal fluid, and dentate gyrus tissue (collectively termed the "*systemic milieu*") across species, we may be able to infer mechanisms that are, ultimately, relevant to the human condition. Specifically, I will investigate which proteins and metabolites of this milieu change from young adulthood to middle age and as a function of exercise (**Figure 1**). If opposing effects of ageing and exercise are found for any of them, they could represent the malleable and prognostic culprits that act through neurogenesis to dictate the fates of our memories. Our first goal was to study the proteins of the dentate gyrus.

In any tissue, there are thousands of proteins, and we want to establish whose levels change with some condition (e.g. exercise), as this could allow us to mimic a desired effect –or reverse an undesired one – by targeting the protein (the basis for much of modern-day pharmacology). This means we will have to *identify* and *quantify* all of them. Because proteins are made up of amino acids arranged in highly complex shapes, it is difficult to study them in their native state. It is easier to first cut them into smaller constituent parts (*peptides*, which are strings of amino acids) with a naturally occurring enzyme called "*trypsin*", identify the *peptides*, and then, piece them together with a computer to identify the *proteins*. The identification is done with a *mass*

spectrometer, a machine that electrically charges the peptides, flies them through an electric field, and breaks them apart into even shorter fragments that can be detected. The peptide sequence and amount can be inferred from the time the process takes and the signal strength. The identified peptides are, then, compared against a digital library to infer the original protein. Finally, statistical models are used to determine which protein levels have changed between conditions (e.g. as a function of aging or exercise).

Using this technique, I found a protein in the dentate gyrus that was responsive to exercise in young adult rodents (i.e. its levels decreased in rats housed with running wheels). From previous studies, this protein is known to be expressed in the dividing cells that give rise to adult-born neurons, and it is situated to regulate cell division in response to signals in the dentate gyrus microenvironment. We are now hoping to examine if genetically manipulating its levels in the hippocampus will change the effect of exercise on neurogenesis and memory. If so, this protein and the factors that regulate it could comprise a druggable mechanism to ameliorate age-related memory decline and, perhaps even, mimic exercise (which could be useful for those physically unable to engage in it). Therefore, it will be important to determine *how* exercise brings about changes in its levels. In the meantime, I have begun studying proteins in cerebrospinal fluid and metabolites in blood from the same rodents, with the hopes of discovering molecules in the circulatory system that could impact neurogenesis and – consequently – memory. Thus far, three metabolites have been identified as changing with age and exercise, one of which increases with exercise and decreases with age. Previous studies suggest that these metabolites could alter neurogenesis. To test this, we will apply them to brain cells in petri dishes and study the growth response. The outcome of the cerebrospinal fluid analysis will indicate if any proteins in circulation display similar trends and whether or not they are related to the candidate protein above through a common mechanism.

In June, I will attend my second Billie Eilish concert; this time in Berlin. I would like for these dear memories not to blur in the future. Until we find the proverbial fountain of youth, I sure hope my blood will let me…

Declaration of Interest

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