Review

Activity-Dependent Induction of Younger Molecular, Cellular and Organism Phenotypes

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Abstract: In several mammalian species including humans, complex stimulation patterns such as cognitive challenge and physical exercise lead to improvements in organ function, organism health and performance, as well as possibly longer lifespans. The hypothesis is presented here that activity-dependent transcriptional programs, induced by these environmental stimuli, temporarily and lightly de-differentiate somatic cells such as neurons and muscle cells into a state that resembles functionally younger cells to allow cellular remodeling and adaptation of the organism to environmental change. This cellular adaptation program targets several process classes that are heavily implicated in aging, such as mitochondrial metabolism, cell-cell communication, intracellular signaling and epigenetic information processing and leads to functional improvements in these areas. I reverse engineer these activity-dependent gene programs, identify critical molecular nexus points such as CREB, MEF2 and cFos and speculate as to how one might leverage them to prevent and attenuate human aging-related decline of body function, enhance human performance and restore more youthful levels of function and morphology. The findings presented here can serve as a basis for the study and development of effective longevity efforts as the underlying gene programs could be used as markers for treatment success and as targets for therapy development.

Keywords: cell; biology; aging; medicine; transcription; activity; neurons; brain; immune system; muscle

1. Introduction

One potential way of achieving longer life- and healthspans as well as performance increases in humans is the induction of a cell state in various tissues that is normally found in younger individuals. The induction and maintenance of a younger cell state might enable the restoration and maintenance of youthful body function and morphology as well as increase adaptation capabilities. As such, induction of a functionally younger cell state will be central to efforts in human life extension, health and performance enhancement and for life under hostile conditions (e.g. during spaceflight and extraterrestrial colonization). An ever-growing list of studies shows that mammalian aging, as defined by certain phenotypic organism changes over time, can be slowed and both health- and life spans can be increased through various interventions such as exercise and nutrition regimens. Yet, the evidence currently presents itself as a patchwork of different findings and hypotheses with few theories probing the logic behind endogenous youthful cell state induction mechanisms. Here, we will investigate activity-dependent gene programs that are activated naturally in various cell types to allow adaptation to changing environmental conditions (i.e. learning, immune defense, muscle strength increase) for their potential to maintain and induce a younger cell state. In order to allow profound adaptation, it seems that many cell types move themselves towards a more immature end of the differentiation spectrum to subsequently redifferentiate, sometimes with different cellular attributes compared to the initial state and oftentimes with an, at least temporarily, younger phenotype (Fig. 1). We will identify concrete molecular targets and approaches that could build the basis for new therapeutic strategies in the field of longevity. We will also speculate as

to how one might optimally leverage activity-dependent gene programs in everyday life to slow age-related decline and possibly regain younger organism function. Throughout the following we will focus first on the central nervous system (CNS) to illustrate the different adaptation and reprogramming mechanisms and then extend the discussion to other organ systems.

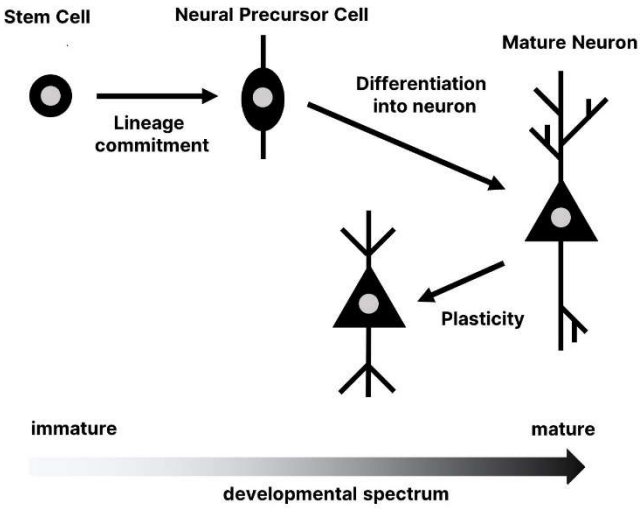

Figure 1. Hypothesis of cellular plasticity as de-differentiation. Strong synaptic stimulation of a mature neuron (right) leads to temporary de-differentiation and movement of the cell state along the developmental axis towards a younger, more immature phenotype. For a while afterwards, many markers of the younger cell state, such as certain epigenetic signatures, improved mitochondrial function and more precise intercellular communication, persist. Continuously repeated activation of this endogenous youthful cell state induction process can lead to a slowing of age-related decline in cell function.

2. The young phenotype and aging

2.1. The young organism

What distinguishes a young animal from an older one of the same species? We can broadly identify two aspects of aging-induced decline: 1) decreased basic function (e.g. slower problem solving, dysfunctional metabolic maintenance, decreased maximum strength) and 2) decreased adaptation capability (e.g. impaired learning of new skills, gaining strength only slowly or not at all after a challenge). Very broadly, and with regard to vertebrates, younger animals have better functioning and more adaptable nervous, motor, immune, reproductive and integumentary systems. We will define what "better" means more precisely below. Concerning brain function as an example, we note the following for mice and humans: better memory, better attention, better extinction of maladaptive behavior such as addiction and better recovery after injuries such as stroke. Most of these are broadly defined at the organism and tissue level. In the brain, a wealth of research has shown that all of these behavioral and tissue properties can be correlated to functions at the cellular level (see (Kandel, 2013) for a general review). For conceptualization, we focus on the cell as a critical organizational unit, while keeping in mind that aging changes can be described at levels ranging from molecules to the whole organism, something we will also do later in our studies here.

2.2. The young cell state

In broad terms, the young cell state can be characterized by 1) higher accuracy, 2) greater flexibility, 3) less harmful contents and 4) characteristic molecular signatures with at least currently somewhat undetermined functional significance. Higher accuracy is found in cellular production and communication mechanisms such as DNA replication and repair, protein turnover, intracellular signal transduction and intercellular

communication. Greater flexibility is expressed in the cellular response to metabolic demands, stress and extracellular signals and allows the cell to adapt to environmental changes. Additional characteristics of a younger cell phenotype are the absence of molecular damage, absence of intracellular aggregates such as prions and iron deposits and expression of characteristic markers such as DNA-methylation signatures. The functional youth of a cell (as opposed to a chronological one) can hence be defined by how much it differs from an older cell with regard to well-defined cellular properties, for instance through the set of all differences in cellular properties between young and old cells. Some of the critical cellular changes in the mammalian brain during aging have been reviewed in (Mattson and Arumugam, 2018) and will also be discussed in the following section. Broadly, they include changes in synaptic signaling, stem cell exhaustion, impaired DNA repair, glia cell activation and inflammation, dysregulated intracellular signaling, mitochondrial dysfunction, oxidative damage, impaired proteostasis and toxin removal and an impaired stress response.

3. Preventing age-related decline through innate mechanisms

With the above notion of differences between young and old cells established, let us see if and how one could stop or delay the transition from the young to old phenotype and even restore more youthful levels in cellular function in old cells. We start with a key observation which is backed by a large body of experimental studies:

The mammalian body has innate mechanisms to protect itself from harm and decline. Surprisingly, these programs must be activated by outside stimuli.

Several studies have shown that physical exercise can protect against cognitive decline in humans and rodents, see reviews in (Lista and Sorrentino, 2010; Mandolesi et al., 2018; Voss et al., 2013) (a more detailed discussion with primary references can be found in a later section). Likewise, mental stimulation such as from enriched environments or cognitive exercise can counteract cognitive decline with age, for reviews see (Leon and Woo, 2018; Mattson et al., 2001; Mora, 2013; Mora et al., 2007). How does the body implement the necessary changes that connect physical or mental exercise to improvements in health? Regarding the mammalian CNS, it has long been known that the activation of many of these innate protection mechanisms depends on activity-dependent induction of defined transcriptional programs.

3.1. The activity-dependent gene program

In neurons, synaptic activity induces gene programs which are under the control of distinct synapse-to-nucleus cascades. Two examples are 1) glutamate-activated synaptic NMDA receptors which induce Ca^{2+}-dependent signaling pathways and activate a unique transcriptional response and 2) BDNF-dependent signaling via activation of TrkB which induces an overlapping genetic program to the NMDA receptor one (for a complete discussion, see for instance (Hagenston and Bading, 2011; West and Greenberg, 2011)). Synaptic activity-dependent genes have been shown to be required for adequate learning and memory and to protect against injury-related damage and neurodegenerative cell death. The genetic program induced by neuronal activity, the activity-dependent gene program (ADGP) is relatively large (>1000 genes) with complex dynamics and a multitude of downstream targets, which we will look at now.

3.2. Activity-dependent transcription factors and immediate early genes

The first group of processes and genes is that of activity-dependent transcription factor (ADTF) activation (i.e. CREB and MEF2) and immediate early gene (IEG) transcription (i.e. cFos, Egr1, Arc, Npas4). The main functions of these rapidly activated programs (minutes to hours) are to coordinate transcription of downstream genes and execute immediately necessary changes to cell physiology. Activation of these cascades and genes is reduced in aged animals and exogenous upregulation of many of these genes, either

directly or indirectly, protects against age-related functional decline. CREB for instance is a central hub for activity-dependent transcription. In one study, young animals showed higher CREB phosphorylation and higher expression levels of cFos and Egr1 in the dentate gyrus and better memory performance than older animals (Villeda et al., 2014). Transfusion of young blood into old animals improved these molecular and behavioral parameters significantly. Yu et al have shown that CREB overexpression in the hippocampus can rescue aging-associated memory deficits in rats(Yu et al., 2017), presumably through changing critical synaptic parameters. CREB has also been shown to mediate caloric restriction-induced rescue of age-related cognitive impairment (Fusco et al., 2012) and CREB levels were diminished in a mouse model of Alzheimer's disease (Pugazhenthi et al., 2011). Barker et al have shown that in the brain the activity-dependent transcription factor Mef2a/c is recruited through exposure to enriched environments and its overexpression protects against Alzheimer-like cognitive impairment in mice (Barker et al., 2021). Qiu et al. report that expression of Npas4 and Arc in the hippocampus is reduced in aged memory-impaired animals (Qiu et al., 2016). Myrum et al show increased baseline Arc levels in the hippocampus of aged memory impaired animals, whereas Egr1 and cFos induction by a novel environment were weakened (Myrum et al., 2020). Haberman et al report increased levels of hippocampal cFos in aged memory-impaired animals. Penner et al and Desjardins et al revealed that expression of Egr1 is reduced in the hippocampus of aged animals (Desjardins et al., 1997; Penner et al., 2016). Expression of activity-induced Dnmt3a2 in the hippocampus is reduced in old animals and Dnmt3a2 overexpression in old animals restores memory function to young levels (Oliveira et al., 2012; Oliveira et al., 2016). Homer1a is reduced in aged animals and has been correlated to memory performance (Kaja et al., 2013). Chatzi et al. have found that in mice physical exercise in the form of running in a wheel, lead to substantial upregulation of cFos in the hippocampus with subsequent changes in neuronal function (Chatzi et al., 2019), a result which might explain observations of exercise improving neural plasticity and cognitive function in humans. Several immediate early genes (i.e. Npas4, Atf3, Ptgs2) were also shown to confer protection against neuronal cell death in animal stroke models (Zhang et al., 2009). Overexpression of CREB has been shown to upregulate death-inhibition genes and exert a protective effect in mouse neurons (Tan et al., 2012). One of the defining characteristics of all of these genes is that they can be induced by naturally occurring stimuli (e.g. novel environments or potential mates). We can hence make three observations: 1) IEG baseline expression and induction are altered in older animals, 2) overexpression of IEGs and ADTFs in older animals leads to functional improvements and performance comparable to young animals, 3) treatments that enhance function in older animals to young levels also increase IEGs. IEGs and their induction mechanisms are hence heavily implicated in aging and mechanisms and the transition to a younger cell phenotype.

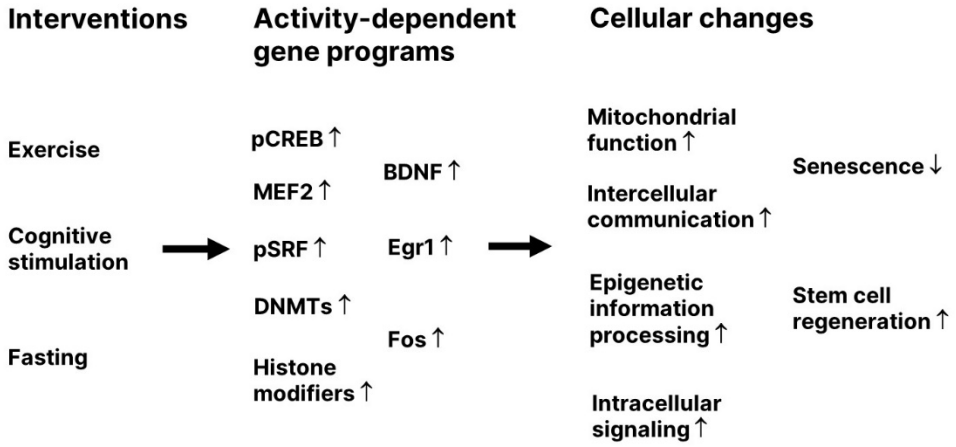

Figure 2. Activity-dependent gene programs connect health- and longevity- promoting interventions to beneficial cellular changes. Interventions such as exercise, cognitive stimulation or fasting induce activity-dependent gene programs in a variety of cell types. These programs include transcription factors such as CREB, epigenetic regulators such as DNA-methyltransferases (DNMTs) and histone modifiers, as well as immediate early genes. These programs then coordinate adaptation and remodeling processes that translate to a functionally younger cell phenotype.

3.3. Metabolism and cell survival

One functional group that is very important with regard to longevity and aging is metabolism, with a focus on mitochondrial physiology. Mitochondria are crucial components for cellular energy supply, but also exert damage in the form of reactive oxygen species and free radicals and are crucial parts of apoptotic pathways. The neuronal activity-dependent gene program contains many genes that regulate mitochondrial function. The pro-apoptotic mitochondrial calcium transporter MCU has been shown to be downregulated by neural activity via Npas4 induction, a mechanism which protects neurons against NMDA-induced cell death (Qiu et al., 2013). Npas4 in turn has been shown to protect against ischemic cell death in the brain *in vivo* (Zhang et al., 2009). Synaptic activity-dependent gene programs were shown to induce genes that promote a neuronal Warburg effect (e.g. Glut3, Mct1) (Bas-Orth et al., 2017), which allows neurons to use anaerobic glycolysis, presumably to meet increased energy demand during intense neuronal activity or possibly to avoid excessive ROS production. Lau et al have shown that the pro-apoptotic Bcl-2-associated gene bbc3 is downregulated by neuronal activity (Lau and Bading, 2009). In line with these studies, Gopalakrishnan et al report in a murine cell line that CREB regulates Cytochrome c transcription (Gopalakrishnan and Scarpulla, 1994). We thus see that activity-dependent transcription can alter mitochondrial physiology and antagonize apoptotic signaling pressure.

3.4. Intercellular communication

Another factor that is influenced by activity-dependent transcription is cell-cell communication, in the case of neurons usually via synapses. Previous research has shown that aging is accompanied by changes in synaptic function and plasticity (Bartsch and Wulff, 2015; Burke and Barnes, 2006; Buss et al., 2021; Kumar and Foster, 2007; Temido-Ferreira et al., 2019), which has been proposed to explain changes in mental function and cognitive decline. In neurons, one important determining factor of intercellular communication is synaptic NMDA receptor composition. Most adult forebrain neurons express the obligatory subunit NR1 together with the NR2A subunit. During development in rodents and humans, most forebrain neurons display NR1 together with NR2B, before switching to NR2A in the adult (Bagasrawala et al., 2017; Zhang et al., 2015). Adult synapses that contain higher NR2B levels, thus mimicking an embryonic or postnatal developmental state, were shown to undergo more pronounced structural plasticity (Barria and Malinow, 2002), in line with neurons in young animals needing higher plasticity levels to implement circuit remodeling during development. Importantly, it has been shown that Grin2b upregulation and Grin2a downregulation in mature neurons can be induced by neuronal activity, thus mimicking a younger phenotype in older neurons (Lissek et al., 2021). Another important class of activity-dependent intercellular signaling molecules is that of neurotrophins, which are involved in neuronal circuit wiring during development. A representative of this class is BDNF, which is regulated by many of the mechanisms discussed above such as CREB phosphorylation and Npas4 binding and modulates a variety of neuronal functions including synaptic plasticity, spine density and metabolism. Studies have shown that BDNF concentrations decrease in the brain with age in rodents (Hattiangady et al., 2005; Molinari et al., 2020; Silhol et al., 2005) and humans (Budni et al., 2015; Erickson et al., 2012; Molinari et al., 2020; Oh et al., 2016; Romanczyk et al., 2002; Tapia-Arancibia et al., 2008). Artificially enhancing BDNF levels could rescue age-related microglia dysfunction (Wu et al., 2020) and overexpression of BDNF in the hypothalamus was shown to promote healthy metabolic aging (McMurphy et al., 2019). Increasing BDNF levels in

the hippocampus was reported to protect against effects of chronic stress (Taliaz et al., 2011) and overexpression of BDNF was shown to protect against AD phenotypes in mice and primates (Nagahara et al., 2009). Fasting restores BDNF levels to healthy amounts in HD mice (Duan et al., 2003). However, other studies have shown that broad overexpression of BDNF in the forebrain of wild type animals can have many negative effects such as increased anxiety and seizures (Papaleo et al., 2011) and memory-impairments (Cunha et al., 2009). For a comprehensive review on BDNF in the aging brain, see (Miranda et al., 2019). With the two examples of a changed NR2A/NR2B ratio and an increase in BDNF signaling, we see that neuronal activity can lead to a transient transition to a younger cell state, inducing phenotypes that are normally present in younger animals.

3.5. Senescence

One central hallmark of cellular senescence is stable cell-cycle arrest of proliferating cells (Campisi and d'Adda di Fagagna, 2007). As neurons are postmitotic, this notion becomes inadequate in describing senescence for this cell type. Nevertheless, previous studies found that neurons in culture developed senescent features including a senescence-associated secretory phenotype and dysfunctional autophagy (Moreno-Blas et al., 2019). Molofsky et al have shown in mice that aging reduces progenitor proliferation in the subventricular zone and neurogenesis in the olfactory bulb and that this reduction is diminished in $p16^{INK4a}$ deficient mice (Molofsky et al., 2006). Jurk et al have described a p21-mediated senescence-like phenotype in neurons including high ROS production and oxidative damage, IL-6 production and β-galactosidase activity with short-term caloric restriction reversing some age-dependent increases in biomarkers (Jurk et al., 2012).

3.6. Neurogenesis

Another important function that is diminished with aging is tissue regeneration through cell replacement. The underlying mechanism of stem cell exhaustion is observed in many tissues and causes progressive decline in organ function. In the brain, this happens via neurogenesis and in adult humans neurogenesis has been reported in the hippocampus throughout aging (Boldrini et al., 2018) (Moreno-Jimenez et al., 2019), for review and outstanding questions see (Gage, 2019). CREB signaling has a profound effect on adult hippocampal neurogenesis, for review see (Ortega-Martinez, 2015). Nakagawa et al showed that CREB regulates adult hippocampal neurogenesis in mice (Nakagawa et al., 2002), a finding reproduced by Fujioka et al (Fujioka et al., 2004). BDNF, another central ADG, has been reported to stimulate hippocampal neurogenesis in adult rats if infused (Scharfman et al., 2005) and Kwon et al reported that treadmill exercise induces phosphorylation of ERK1/2 and CREB and induces BDNF in the rat hippocampus and cortex (Kwon et al., 2013). Activity-dependent signaling via CREB and BDNF is thus shown to enhance neuronal stem cell proliferation to more youthful levels.

3.7. Proteostasis

Proteostasis (i.e. the regulation of protein turnover) is an essential component of healthy neuronal function and has been shown to be affected by aging in general (Kaushik and Cuervo, 2015; Meller and Shalgi, 2021) and in brain aging in particular (Hetz, 2021). An important function of proteostatic mechanisms is the removal of misfolded proteins. ATF4 (also known as CREB2) is a transcriptional repressor that is inhibited in neurons by neural activity (Bartsch et al., 1995) and which has been shown to regulate genes involved in protein synthesis and UPR functions (Han et al., 2013), with its overexpression resulting in increased protein synthesis, oxidative stress and cell death. Neural activity is thus presumed to partly inhibit cell death via CREB2 downregulation-mediated proteostasis.

3.8. Epigenetics

Epigenetic remodeling is a central aspect of activity-dependent gene programs in neurons and is crucially involved in brain aging. In neurons, neural activity leads to acute

changes in histone modifications and histone shuttling (Chawla et al., 2003), DNA methylation (Guo et al., 2011; Halder et al., 2016) and non-coding RNA transcription (Kim et al., 2010). Peleg et al reported that altered histone H4K12 acetylation was associated with failed induction of learning-associated gene expression in the aged mouse brain and memory deficits (Peleg et al., 2010). Reversal of H4K12 acetylation lead to rescue of cognitive faculties. Benito et al found in mice that HDAC inhibition via suberoylanilide hydroxamic acid (SAHA), also known as vorinostat, rescued age-dependent spatial memory decline (Benito et al., 2015). However, Castellano et al found no evidence of an effect on memory through HDAC inhibition (Castellano et al., 2014). Cosin-Tomas et al found that in the senescence mouse model SAMP8, mice displayed a range of epigenetic alterations as compared to wildtype controls and that these changes were reversed by exercise (Cosin-Tomas et al., 2014). Han et al demonstrated an increase in H3K4 methylation in the macaque brain during aging (Han et al., 2012). Morse et al reported in rats that histone methylation patterns were changed during aging and that exposure of aged animals to enriched environments lead to a reversal of these changes and rescued memory deficits (Morse et al., 2015). Lu et al demonstrated aging-dependent changes in DNA methylation in the human brain (Lu et al., 2017) and Masser et al also reported different methylation patterns in the hippocampus between young and old mice (Masser et al., 2017). Oliveira et al found that overexpression of the neural-activity regulated DNA methyltransferase Dnmt3a2 was able to rescue aging-induced cognitive impairment (Oliveira et al., 2012; Oliveira et al., 2016). Ianov demonstrated aging-dependent changes in methylation patterns of synaptic genes in rats (Ianov et al., 2017). Lubin et al showed that DNA methylation changes in BDNF are correlated to its activity-induced expression and that blockade of DNA methylation impaired memory formation in mice (Lubin et al., 2008). Penner et al have reported that in the hippocampus of mice, aging-dependent reduction in the expression of the IEGs Arc and Egr1 after behavioral challenge was associated with changes in the methylation patterns in these genes (Penner et al., 2016; Penner et al., 2011). Another epigenetic regulation mechanism is transcription of microRNAs which are small RNAs that do not code for proteins but can bind to mRNAs and lead to their degradation. In the brain, several miRNA have been shown to be regulated by neuronal activity (Sim et al., 2014). miRNA-132 has been shown to be reduced in neuronal stem-cells in Alzheimer's disease and its upregulation rescued memory deficits in an AD mouse model (Walgrave et al., 2021). Importantly, Vo et al showed that miRNA-132 is induced by activity-regulated neurotrophins such as BDNF (Vo et al., 2005) and Wayman et al showed that miRNA-132 is regulated by neural activity in an NMDAR-, ERK1/2- and CAMKII/IV-dependent manner (Wayman et al., 2008). Nudelman et al extended these findings to living animals by showing that miRNA-132 is induced in the murine hippocampus by contextual fear learning (Nudelman et al., 2010). We make two important observations: 1) Aging is associated with profound and widespread epigenetic alterations, 2) these alterations can be reversed or counteracted, at least temporarily, by endogenous activity-dependent signaling.

3.9. Intracellular signaling

Another function that is affected by aging is intracellular signaling, especially membrane-to-nucleus signaling. Many of the cascades involved have protein kinases or phosphatases as central hubs and we will focus on those here but will only give a few example references for each because treating the whole literature would go beyond the limits of this paper. Key upstream players in neuronal aging are Ca^{2+}-signaling (Chandran et al., 2019) and cAMP-related signaling (Di Benedetto et al., 2021; Kelly, 2018). Fukushima et al have found in mice that CaMKIV expression is reduced in aged mice and that overexpression of CaMKIV in aged animals rescues memory impairments (Fukushima et al., 2008). Ramos et al have shown that inhibition of PKA improves age-related cognitive dysfunction in rats and monkeys (Ramos et al., 2003), a finding replicated in flies by Yamazaki et al (Yamazaki et al., 2010). Karege et al have shown that PKA activity declines with age in

the hippocampus and cortex of rats (Karege et al., 2001). One study has found age-related impairment in brain ERK1/2 phosphorylation (Zhen et al., 1999). P38 signaling is pro-apoptotic and has been linked to cognitive decline in Alzheimer's mouse models (Kheiri et al., 2018). Calcineurin (CaN) expression is increased in the aged rodent brain and leads to CREB dephosphorylation (Foster et al., 2001). CaN might also mediate AD changes (Hopp et al., 2018) and human cognitive impairment (Mohmmad Abdul et al., 2011). PKC activity has been proposed to contribute to decline in cognitive performance and inhibition of PKC lead to a rescue of aging-dependent memory impairments (Brennan et al., 2009). These findings have also been replicated in stress-related cognitive dysfunction (Hains et al., 2009). Research has also shown that expression of certain PLC isoforms in the rat brain decreases with age (Shimohama et al., 1998) and suggested that activation of PLC could perhaps enhance protection against Alzheimer's associated phenotypes (Magno et al., 2019). Lin et al have shown that swimming exercise in rats activates PI3K/AKT signaling and attenuates aging-associated cognitive decline (Lin et al., 2020).

4. The logic of innate induction of younger cell phenotypes

4.1. Energy conservation and stimulation frequency

A question presents itself now: Why doesn't the body activate the above programs periodically to keep itself healthy, even in the absence of outside stimuli? One obvious explanation could be energy conservation and the principle "only adapt when necessary". Humans may have evolved in environments where energy supply in the form of food was scarce, hence cells match expenditure to requirements. In addition, in our potentially more stimulating ancient environments, physiological challenge was so pervasive that there was no need for an intrinsic induction of molecular adaptation mechanisms, as the outside world lead to activation of cellular adaptation mechanisms periodically.

4.2. Coordination

Another reason for restricting cellular plasticity programs is to allow time for coordination of subsequent adaptive changes within cells and across tissues in the body. Most complex and strong stimuli induce adaptations in several tissues at the same time (i.e. brain, skeletal muscle, heart muscle, endocrine system). Within each tissue, these adaptations take time. It could hence be that discontinuous engagement of cellular plasticity mechanisms is a prerequisite to allow for body-wide coordination across tissues.

4.3. Limiting downsides

Another possible explanation for restricted ADGP activation is that these programs might have negative consequences outside of an increased energy demand. An example could be that certain cellular processes will be occupied for remodeling tasks and hence unable to perform more acute functions. If ribosomes are occupied for protein production to allow remodeling of the cytoskeleton, these ribosomes will not be able to translate at high capacity synaptic proteins in response to synaptic stimulation.

5. Engineering the activity-dependent gene program to extend health- and lifespan

We have so far learned that the body possesses intrinsic mechanisms to combat aging-related changes and that these mechanisms can be activated by environmental stimuli. We have also dissected the details of the underlying molecular programs. Now, we try to leverage these insights to construct a framework for engineering human longevity interventions.

5.1. Critical nodes

One of the first central aspects when leveraging intrinsic cellular mechanisms to induce a younger cell phenotype is to identify promising attack points. One could of course try to target all hallmarks of aging independently (e.g. use one pharmacological compound for mitochondrial metabolism, another one for protein turnover and so forth) but

this strategy seems misguided for two reasons: 1) It is not very economic, necessitating the use of multiple treatment entities with an unknown upper bound on the number of interventions, 2) We currently most likely don't even know all aging endpoints we would have to correct. Hence, it might be advantageous if we found critical nodes that have a global regulatory function and provide unusually potent means of cellular remodeling. Two molecule classes seem amenable to this strategy: 1) transcription factors, 2) signaling molecules such as intracellular kinases or cell-surface receptors. A transcription factor-based strategy is supported by evidence from cellular reprogramming techniques where overexpression of a few transcription factors leads to a profound change in cellular identity and physiology. One example is reprogramming of fibroblasts into neurons via overexpression of Ascl1, Brn2 and Myrtl1 (Vierbuchen et al., 2010), the other one is production of induced pluripotent stem cells via overexpression of the Yamanaka factors Oct3/4, Sox2 and Klf4 (Takahashi and Yamanaka, 2006). In general both of these techniques use transcription factors with pioneer factor activity (meaning that these transcription factors can access epigenetically "locked" chromatin regions) to profoundly alter the cell state. This brings us to the question of which molecular nodes we should target with our ADGP induction efforts. On the level of transcription factors, there are the central neuronal transcription factors discussed above such as CREB, SRF and MEF2 as well as IEGs such as Fos and Npas4. Evidence for targeting CREB as a nexus point is ample. Increasing CREB activity or levels leads to enhanced memory (Kida, 2012; Sakamoto et al., 2011; Viosca et al., 2009) and protection against neuronal cell death (Tan et al., 2012). However, prolonged caCREB expression also leads to the development of epileptic seizures due to profound network remodeling (Lopez de Armentia et al., 2007) and cognitive aberrations. Npas4 has been shown to protect neurons against degeneration and has also been shown to induce in medium spiny neurons a young transcriptional phenotype of Grin2b upregulation and Grin2a downregulation, as discussed in the section "Intercellular Communication". We can thus identify the following promising nexus points for younger cell state induction based on endogenous signaling factors: CREB, SRF, MEF2, several histone modifiers, as well as Fos, Egr1 and Npas4.

5.2. Metrics

As with most engineering efforts, we need output parameters with which we can evaluate the success of our longevity interventions and subsequently iterate towards better outcomes. One set of markers are macrophysiological ones, which are correlated to health and lifespan in humans such as resting heart rate, heart rate variability, body-mass index and resting blood pressure. Although these are very useful in assessing functional youth, they have several short-comings. An important one is that the measurement of adaptive changes via these parameters takes time because one needs before and after measurements. It would thus be more favorable to have an adaptation marker that can be measured during or immediately after the intervention. A promising alternative are molecular markers. We can leverage our insights about natural genomic longevity programs from above to identify promising biomarkers for cellular adaptation. One pervasive example is activity-dependent transcription factors, such as CREB with phosphorylated serine 133 (S133) and transcription of ADGs such as Fos, Egr1 and BDNF. One could measure these markers after an intervention to immediately gain feedback on the remodeling potency of the stimulus. One possible problem with these markers is that they are usually profiled in the tissues that are directly activated (e.g. brain after learning, muscle after exercise) and hence might be difficult to access. Future studies could investigate whether more accessible cell types or tissues also show differential gene expression or protein activation after stimulation of remote tissues (i.e. immune and blood cells expressing Fos after learning or exercise). A fact that might speak for this strategy is that blood circulates through all parts of the body and comes in contact with all tissues, a strategy which is already being used in liquid biopsies for solid malignant tumors(Heitzer et al., 2019). One could hence use transcription factor activation or IEG transcription as immediately

available adaptation markers that enable faster decision-making as compared to tracking global physiological parameters.

5.3. Dynamics

A critical point in many gene regulatory networks is their dynamic behavior. The components of genetic circuits can combine a variety of basic motifs, such as oscillations, positive and negative feedback, different ramping and decay kinetics into a seemingly unlimited amount of gene expression patterns. In efforts to leverage ADG programs for life extension, these dynamics have to be considered and incorporated into the methods used for impacting the human body. As an example, constitutively active CREB overexpression in the brain has been shown to cause epileptic seizures in mice (Lopez de Armentia et al., 2007). Prolonged overexpression of the Yamanaka factors might lead to creation of cancer cells or dysfunctional cells. In reverse engineering natural longevity programs, we might place special attention on the dynamics of the underlying mechanisms. This is important for practically all interventions such as exercise, pharmacotherapies and gene and cell therapies. If we artificially activate ADGPs via pharmacological stimulation or gene overexpression, we should probably aim to somewhat mimic natural dynamics of these genes so as to not disturb cell physiology too much. Possible downsides of mistiming IEG expression can be inferred from sections "Coordination" and "Limiting downsides" above. It might be that non-natural expression dynamics disrupt intra- and intertissue coordination and that mistimed IEG expression might decrease performance at unwanted times. For exercise and learning, this could mean that mistiming exercise during recovery periods can lead to a reduction or disturbance in cellular reprogramming or, even worse, to cellular damage from overload during critical recovery periods. We should thus pay close attention to the endogenous gene expression dynamics of interventions that are known to prolong life- and healthspan.

6. Interventions

We will now look at which type of interventions we could leverage to impact the longevity gene networks discussed above.

6.1. Exercise

One way to induce activity-dependent transcription in a variety of tissues, including brain and muscle, is physical exercise. Chatzi et al have shown that in mice, running-wheel exercise upregulated the number of cFos-positive cells in the hippocampus by around 2.7-fold and that exercise induced spine formation and an increase in EPSC amplitude in neurons (Chatzi et al., 2019). Farmer et al found in rats that voluntary exercise induced expression of BDNF, NR2B and GluR5 in the dentate gyrus and induced synaptic plasticity (Farmer et al., 2004). Chen et al describe an upregulation of S133-CREB phosphorylation and an upregulation of BDNF transcription in the mouse hippocampus after exercise (Chen and Russo-Neustadt, 2009). Similarly, Shen et al have found that exercise increased phosphorylation of CREB and MAPKs p42 and p44 (Shen et al., 2001). Kwon et al show that treadmill exercise induces ERK1/2 and CREB phosphorylation and BDNF induction in the rat hippocampus and cortex (Kwon et al., 2013). Vaynman et al report hippocampal upregulation of pCREB and BDNF in rats after exercise (Vaynman et al., 2003). Lin et al showed that running exercise increased AKT, PKC and ERK phosphorylation in the hippocampus of wildtype and transgenic Alzheimer's mice and that it attenuated neurodegeneration (Lin et al., 2015). In humans, serum BDNF levels are increased after exercise (Saucedo Marquez et al., 2015; Schmolesky et al., 2013; Slusher et al., 2018) and blood analysis studies suggest that exercise induced BDNF release in humans happens in the brain (Rasmussen et al., 2009; Seifert et al., 2010). Interestingly, studies in human subjects have shown that chronic exercise decreases resting serum BDNF levels (Babaei et al., 2014; De la Rosa et al., 2019; Nofuji et al., 2008). One very interesting study connects exercise to brain-BDNF release and IEG induction via FNDC5 and shows that

AAV-mediated FNDC5 overexpression in the liver of mice can lead to hippocampal gene induction, including BDNF (Wrann et al., 2013). Another major way of how exercise promotes cognitive function seems to be stimulation of adult neurogenesis in different mammals, see (van Praag, 2008) for review. Deficiency in cellular replacement has been proposed to be a major player in aging and numerous aging therapies target cellular replacement via stem cell injections or stimulation of endogenous stem cell proliferation. In aged mice, running exercise rescues learning performance and enhances neurogenesis (van Praag et al., 2005). If we hence connect our findings of exercise-induced gene programs to our discussions about intrinsic adaptation mechanisms, the following picture emerges. Exercise leads to induction of cellular plasticity mechanisms (i.e. CREB phosphorylation, higher cFos activation levels) and a phenotype which recapitulates a youthful state in aged animals (i.e. Grin2b upregulation, enhanced neurogenesis, increased synaptic plasticity). For humans, this means that it is very likely that physical activity leads to neuronal younger cell state induction and cognitive enhancement. Most animal studies investigate the effects of aerobic activity in the form of running wheel exercises on cognition and health benefits. This focus on aerobic exercise also holds true for most human studies, so it seems that the evidence is currently strong for aerobic exercise, with less available evidence for strength training. This could merely reflect a bias in study design and it is possible that strength training has comparable or possibly even higher benefits. In any case, we note that physical activity is a very effective, low-cost way to induce cellular adaptation programs. New technologies that measure cellular responses to exercise could be leveraged to guide design of personalized exercise regimens.

6.2. Enriched environment and cognitive stimulation

Another intervention that has been shown to protect against aging-related cognitive decline and to lead to memory performance enhancement is environmental enrichment. In animal studies, environmental enrichment is achieved by introducing novel and social stimuli into a housing cage, such as toys, exercise opportunities and mates. In mice, environmental enrichment increases expression of the IEGs Fos, Arc, Bdnf, Atf3, Fosb, Nptx2 and Homer1 among others with a differential pattern according to cell type (Jaeger et al., 2018). Barker et al have demonstrated in the mouse brain that MEF2a/c is recruited through exposure to enriched environments and its overexpression protects against Alzheimer-like cognitive impairment in mice (Barker et al., 2021). Bloodgood et al have found that Npas4 is induced in the mouse hippocampus by environmental enrichment and that it regulates inhibitory signaling (Bloodgood et al., 2013). Zocher et al have demonstrated in mice that environmental enrichment preserves a young DNA methylation signature in the hippocampus during aging (Zocher et al., 2021) and Brown et al report that an enriched environment stimulated hippocampal neurogenesis in mice (Brown et al., 2003). For humans, the environment could hence be a critical factor in inducing activity-dependent gene programs and the induction of a younger cell state. Basic measures could include the creation of a stimulating environment with regular social interactions, a purpose to work towards, a changing scenery and access to green spaces with lots of plants. Kühn et al have investigated what features in an environment promote cognitive health in humans and found that forests, as opposed to cities, promote healthy brain activity (Kuhn et al., 2017). Similarly, previous research has suggested the creation of longevity-ready cities that minimize exposure to harmful influences (i.e. air pollution, heat stress) and provide green spaces to encourage physical activity (Wang et al., 2021). Burzynska et al have investigated the relationship between occupation and healthy brain aging and found that work can protect cognitive health, provided the absence of occupational stressors which can actually worsen cognitive outcomes (Burzynska et al., 2019). Crachiollo et al demonstrate in an Alzheimer's mouse model that cognitive stimulation is more important than physical exercise in preventing cognitive decline (Cracchiolo et al., 2007).

6.3. Nutrition

An interesting way to stimulate at least some of the mechanisms of activity-dependent transcription is intermittent metabolic switching, usually brought on by intermittent fasting. Estrada et al report in mice that fasting leads to an increase in CREB-S133 phosphorylation in the hippocampus, the entorhinal cortex, the arcuate nucleus in the medial hypothalamus and the cortical-amygdala transitional zone (Estrada and Isokawa, 2009). Dietary restriction increases brain BDNF levels in mice (Duan et al., 2003) and BDNF was suggested to mediate increased neurogenesis by dietary restriction (Lee et al., 2002). Stranahan et al also found that fasting increases BDNF levels in the mouse hippocampus which correlates with increased dendritic spine density (Stranahan et al., 2009). Dias et al found that intermittent fasting leads to upregulation of the gene Klotho, better learning and memory and increased hippocampal neurogenesis (Dias et al., 2021). We thus see that intermittent fasting induces activity-dependent gene programs in neurons. Intermittent fasting has been shown in mice to lead to cognitive improvement (Ingram et al., 1987) and neuroprotection (Arumugam et al., 2010; Gudden et al., 2021), effects which are very likely mediated by ADG-induction. Fasting induced ADG programs could hence potentially explain the beneficial effects of intermittent metabolic switching for brain health, as reviewed in (Mattson et al., 2018).

6.4. Limiting and timing of behavioral interventions

In the above discussion, we see that exercise, fasting and cognitive stimulation can all lead to the induction of activity-dependent transcriptional programs with beneficial effects for health and longevity. Yet, in implementing interventions to induce ADG programs, we also have to consider their dynamics and the potential effects of overexertion. Increased neural activity and ADG transcription cause cellular and genomic damage and introduce time-restricted vulnerable and unstable cell states. Neural activity itself for instance increases ROS production and increases mitochondrial DNA damage. The cellular adaptations upon ADG induction, such as heightened transcription and translation might use cellular resources such as ATP and amino acids which are hence not available for other tasks. As another example stands the observation that ADG induction requires DNA double strand breaks (DSBs) (Madabhushi et al., 2015) and that beneficial interventions such as environmental enrichment have been shown to increase neuronal DSB frequency *in vivo* (Suberbielle et al., 2013). In fact, sleep has been tightly linked to DSB repair (Zada et al., 2019) and DSB accumulation during wakefulness has been shown to promote sleep via a Parp1 activity increase in neurons (Zada et al., 2021).

Activity-dependent induction of adaptation mechanisms happens via molecular changes that put the cell in a temporarily vulnerable state. Recovery and correct timing are important to leverage ADGPs for longevity and performance enhancement interventions.

6.5. Gene and cell therapies

A more invasive but also more sustained way of stimulating ADGPs could be achieved through gene therapy approaches. One possibility is to deliver genes encoding for trophic factors either to the tissue of interest or into a peripheral tissue to stimulate ADG transcription. One straightforward approach is overexpression of ADGs directly in the brain, as practiced in many basic studies that are cited in the sections above. Another way is peripheral expression of brain-active factors. A recent study has shown that AAV-mediated delivery of the FNDC5 gene to the liver was able to enhance cognitive performance in a mouse model of Alzheimer's disease (Wrann et al., 2013). A third route to influence ADG expression in target cells could be via CRISPR-based modification of promoter elements to amplify their induction in the desired tissue, for instance through double strand breaks or through the introduction of IEG-based transcriptional amplifiers such as cFos promoters, E-SARE (Kawashima et al., 2013) or RAM (Sorensen et al., 2016). One can also imagine the delivery of engineered cells that release trophic factors or directly stimulate target cells via cell-cell contacts. Glial cells or neurons could be transplanted and

act as "ADG pacemakers", periodically stimulating ADGPs and thus promoting adaptation in target cells. Approaches to engineer neural cells have been outlined previously and could involve the creation of highly specialized genomically engineered cells that serve a very defined purpose (Lissek, 2017).

7. Other Tissues

We have treated the ADG program with the example of neuronal cells above. In the following passages, we will give an overview of how these findings relate to two other organ systems that are affected by aging, namely the muscle and immune systems.

7.1. Muscles

Skeletal muscle cells display a very similar ADG program to that of neurons, which is not surprising since muscle cells share many of the same features as neurons, such as electrical stimulation sensitivity and a focus on Ca^{2+} related intracellular signaling mechanisms. Similar to neurons, depolarization and synaptic activation induce signaling molecules such as CaMKIV, CREB and MEF2, usually in the same directions as in neurons, with similar downstream adaptations in mitochondrial function, intracellular signaling and transcriptomic changes. These signaling mechanisms and adaptive molecular and functional changes have been reviewed extensively by Egan et al (Egan and Zierath, 2013). The implications are also similar, namely that repeated activity-dependent activation of these mechanisms by for instance exercise can contribute to an induction of a younger cell state and possibly explain resulting metabolic changes such as restored insulin sensitivity.

7.2. Immune system

Similar to neuronal and muscle cells, immune cells display activity-dependent transcription mechanisms and again with very similar induction mechanisms focusing on Ca^{2+} signaling (Feske, 2007; Monaco et al., 2016). Just as in neuronal and muscle cells, immune cells activate prototypical parts of the ADG program such as CREB (Wen et al., 2010), Fos (Patil et al., 2013), and Egr1 (McMahon and Monroe, 1996) among many others, with notable importance of calcineurin due to the clinical relevance of calcineurin inhibitors such as tacrolimus in immunosuppression. An interesting question that arises is how ADGPs are endogenously activated in immune cells and how one could do so deliberately. Apart from activation by pathogen components, immune cells are activated by synaptic connection onto each other, i.e. "immunological synapses" (Dustin, 2014). This raises the question of whether and how one could activate these mechanisms to induce a younger immune cell phenotype. One way to do so indirectly might be, similar to neurons and muscle cells, physical exercise, as exercise has been implicated in immune system adaptation (Nieman and Wentz, 2019; Tylutka et al., 2021).

8. Considerations and caveats

8.1. Advantages of using intrinsic longevity programs

A clear advantage in using intrinsic mammalian longevity programs such as the ADG program is that nature has already solved the problem of how to coordinate the required physiological changes to make an animal live longer. Regarding primarily human-designed therapies such as pharmacological compounds targeting single metabolic pathways or gene therapies targeting only mitochondria, we have very limited insight in the dynamics of how to induce changes and at what time-points. Indeed, as many longevity interventions might necessitate tightly coordinated communication between cell types and organ systems, the underlying engineering efforts might be hindered by the complexity of the mechanisms involved. Against this notion, as we have discussed above, stands the surprising finding that at least at the cellular level, static expression of relatively few molecules, such as the Yamanaka factors Klf4, Sox2, Oct-3/4 and c-Myc, can induce highly specific cell identity changes and movement along the differentiation spectrum. It might

hence be a viable strategy to identify and manipulate critical signaling nodes that have the capacity to implement widespread, cross-tissue changes.

8.2. Animal studies

One central point to consider is that many of the studies cited above were performed in non-human model organisms, usually mice or rats. It might be that ADGP activation and subsequent cellular adaptation happen differently in humans, if at all. An interesting case in point is the human capacity for adult neurogenesis in the brain. While many studies have replicated this phenomenon in mice, there is ongoing debate on whether and to what extent neurogenesis happens in adult humans (Gage, 2019). Many studies have shown that crucial parts of the ADG program are conserved in humans, with interesting human-specific elements being involved in brain function (Hardingham et al., 2018; Pruunsild and Bading, 2019). Indeed, studies have shown that many ADG induction mechanisms are conserved in human iPSC-cell neurons, such as differential induction of Npas4 by Ca^{2+}-elevating inputs but not those which stimulate cAMP production (Lissek et al., 2021).

8.3. Magnitude of the effects

An important caveat concerns the magnitude of effects that can be achieved. Could we produce supraphysiological adaptation effects or will we have to work within limits that are currently implied by how much exercise, nutrition and cognitive activity slow the aging progress? After all, so far there are no reports that behavioral modifications could extend lifespan beyond 120 years at maximum. Could we theoretically reach human lifespans beyond 200 years by modifying ADGP induction? I cannot currently see an obvious reason why not. It is however questionable whether we could do so with a regular human lifestyle. As mentioned above, ADGP induction and subsequent cellular remodeling lead to vulnerabilities in cell physiology that necessitate a recovery period. Perhaps a very profound or long-lived ADGP induction would necessitate a period of lowered metabolism, such as torpor, to allow for the necessary recovery and cellular remodeling.

9. Conclusion

With activity-dependent gene inductions, nature has solved the critical problem of how to adapt an organism to a changing environment over the long-term. Interestingly, these gene programs move the cell to a younger cell state along the differentiation spectrum. It seems that this cellular adaptation effect is relatively mild, presumably to tightly match energy expenditure to requirements and to limit cellular vulnerability during remodeling. ADG program components could be used as biomarkers for adaptation during various interventions and can be concrete targets for efforts in combating age-related health effects. Promising components that are present in practically all tissues include signaling nexus points such as the activity-activated transcription factors CREB, SRF, MEF2 and IEGs such as Fos, Egr1 and BDNF. These components could be used to measure and titrate interventions such as exercise or nutritional programs to ensure optimal dosing and timing for health and performance enhancement, as well as longevity efforts.

Author Contributions: TL wrote the paper.

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