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Review

The 'middle-aging' brain

Sebastian Dohm-Hansen, [1,2,3] Jane A. English, [1,2] Aonghus Lavelle, [1,3] Carlos P. Fitzsimons, [4] Paul J. Lucassen, [4] and Yvonne M. Nolan [1,3,*]

Middle age has historically been an understudied period of life compared to older age, when cognitive and brain health decline are most pronounced, but the scope for intervention may be limited. However, recent research suggests that middle age could mark a shift in brain aging. We review emerging evidence on multiple levels of analysis indicating that midlife is a period defined by unique central and peripheral processes that shape future cognitive trajectories and brain health. Informed by recent developments in aging research and lifespan studies in humans and animal models, we highlight the utility of modeling non-linear changes in study samples with wide subject age ranges to distinguish life stage-specific processes from those acting linearly throughout the lifespan.

Highlights

Middle age (roughly the fifth and sixth decades of human life) has historically been understudied compared to more advanced age, but is emerging as marking a possible shift in brain aging that is predictive of future cognitive health, while still being amenable to intervention.

Some age-related changes of the brain display approximately linear trajectories throughout adult life, whereas others change non-linearly in a life stage-specific manner. Middle age features accelerating changes in gait, reaction time, memory, functional connectivity, white matter integrity, and neuroinflammation, as well as in gene and protein expression.

Aging processes in systems and organs other than the brain (such as the systemic circulation) impact upon brain aging and could even accelerate it.

In females, menopause is a defining feature of middle age that is associated with non-linear changes of the brain, some of which have implications for cognitive aging.

Middle age as a possible turning point in brain aging

Although public conceptions vary, middle age is commonly taken to mean the ages between 40 and 60–65 years, and does not merely represent a 'mid-point' of the lifespan but is a perceived transition period to 'old age' [1]. From a health science perspective, middle age has been argued to be a period prognostic of future health [2–5]. Nevertheless, middle age has remained understudied compared to advanced age, when effects might be greater but interventions may be too late or have reduced effectiveness. As it pertains to the brain, middle age is associated with specific and modifiable risk factors for future dementia risk [6] (Box 1). These are important observations because the global population aged 60+ years is set to double by the year 2050, which will entail concomitant increases in case numbers of dementia and significant hardship for patients and caregivers [7]. Although aging is naturally associated with a degree of cognitive decline, there is considerable interindividual variability. We highlight the aging processes of the brain that could be especially relevant during middle age and prove to be prognostic of future cognitive outcomes.

Recent empirical work suggests that biological aging is neither a linear process that is consistent across individuals [8–11] nor does it proceed at the same rate throughout the body for any given chronological age [12–14]. Instead, it is thought to comprise many cell-, tissue-, and organ-specific subprocesses that can interact systemically and are driven by internal and external insults operating at multiple levels of biology [15–17]. Tissues may even go through different aging phases across the lifespan, each defined by specific molecular signatures and temporal dynamics, some of which are non-linear [10]. One such period in life appears to be the transition from middle to older age [10,18]. In the human brain, the expression of genes and proteins related to synaptic, inflammatory, and longevity-associated pathways such as mammalian target of rapamycin (mTOR) has been found to undergo accelerating or abrupt changes during this transition [18–22], and studies in rodents have reported similar non-linearities during midlife [12,13,23,24]. In females, some of these effects may be related to the complex changes of menopause [25]. These findings are particularly interesting in light of large neuroimaging studies that show accelerating structural and functional changes during middle age [26–29], some of which have been found to be

[1]Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland
[2]INFANT Research Centre, University College Cork, Cork, Ireland
[3]APC Microbiome Ireland, University College Cork, Cork, Ireland
[4]Swammerdam Institute for Life Sciences, Brain Plasticity Group, University of Amsterdam, Amsterdam, The Netherlands

*Correspondence: y.nolan@ucc.ie (Y.M. Nolan).

Box 1. Middle age lifestyle and modifiable (risk) factors

Although biological aging may be inevitable, several of its underlying processes are likely to be modifiable and might be targeted to attenuate functional decline. For instance, lifestyle factors such as tobacco smoking, alcohol consumption, and diet have been associated with changes in neuroimaging-derived estimates of brain aging from middle to old age [30,122]. In addition, some risk factors for dementia, including hypertension, obesity, and alcohol consumption, have been identified by the 2020 *Lancet* Commission report on dementia prevention [6] as being especially relevant during middle age and proposed as life stage-specific modifiable risk factors. Because individual longitudinal trajectories of cognitive change during middle age are highly variable and are partly independent of early-life and congenital factors [5,31], this period could serve as a point of intervention.

As an example, physical activity has salutary effects on several body systems, and these likely extend to the brain and cognition. Meta-analyses of exercise (especially aerobic) interventions have suggested that exercise may slow down age-related volumetric loss in the left hippocampus of healthy older adults [123] and improve various measures of white matter integrity in frontal and temporal lobe tracts across the adult lifespan, including during middle age [124]. This is particularly relevant in light of the accelerating decline in hippocampal volume [63] and associational white matter volume [67] beginning in midlife. Relatedly, higher levels of physical activity are associated with decreased longitudinal declines in episodic memory in middle-aged and older adults [125,126]. In addition, the protective effects of educational attainment and social contact frequency on cognition appear to be partly explained by an association with higher levels of self-reported physical activity [125,126]. Such findings could prove useful in public health efforts to prevent dementia. As a caveat, a review of 24 meta-analyses found that the effects of various forms of exercise on cognition, irrespective of age range, were inconclusive and possibly subject to publication bias [127]. Moreover, there is evidence to suggest that the relationship between cardiorespiratory fitness and cognitive function at age 38 years may be accounted for by childhood cognitive scores [128]. This highlights the need for further studies on the longitudinal effects of exercise interventions on cognitive aging.

Finally, current geroscience endeavors to identify common molecular and cellular pathways that drive organismal aging [129]. By targeting identified 'hallmarks of aging' [78] with pharmaceutical compounds (e.g., metformin, senolytics, and rapamycin), clinical studies at various stages are underway in an effort to combat dementia and neurodegeneration, although these are mainly in older adults and have had varying levels of success [130–132].

predictive of future cognitive decline [5,30,31] and may be driven by molecules in the systemic circulation [32–35].

We review here emerging evidence at multiple levels of analysis (ranging from behavior to molecules), from both human and rodent studies, suggesting that middle age could mark a shift in brain aging that is driven by central and peripheral processes with relevance for future cognitive health. We argue that applying recent trends in aging research to this period of life could reveal novel biomarkers and possible interventions to combat cognitive decline in an increasingly older global population. We emphasize the utility of non-linear analysis approaches in combination with a wide subject age range to better disentangle life stage-specific changes from lifelong aging biomarkers (Figure 1).

Cognitive changes of the middle-aged brain

A lesson from the longest-running study of aging in the USA (the Baltimore Longitudinal Study of Aging, beginning in 1958) is that physiological and neuroimaging phenotypes often show highly divergent and non-linear relationships with age [36]. Therefore, in attempting to identify cognitive functions that could be particularly vulnerable at any given life stage, we might look for evidence of accelerating change (Box 2). Most cognitive domains display a roughly linear trajectory of decline that begins in one's late 20s, but for some domains, including memory, reasoning, measures of reaction time, and the effect of cognitive load on gait, the decline seems to accelerate, especially toward the end of middle age [37–40]. Notably, in the transition from middle to old age, longitudinal trajectories of episodic memory (i.e., memory for everyday events) appear to be particularly unstable, suggesting that the performance of a person can start to change abruptly, which may reflect an increasing environmental influence [41]. Indeed, rapidly evolving social relationships (e.g., parental loss and family obligations) and interindividual variability in career trajectories

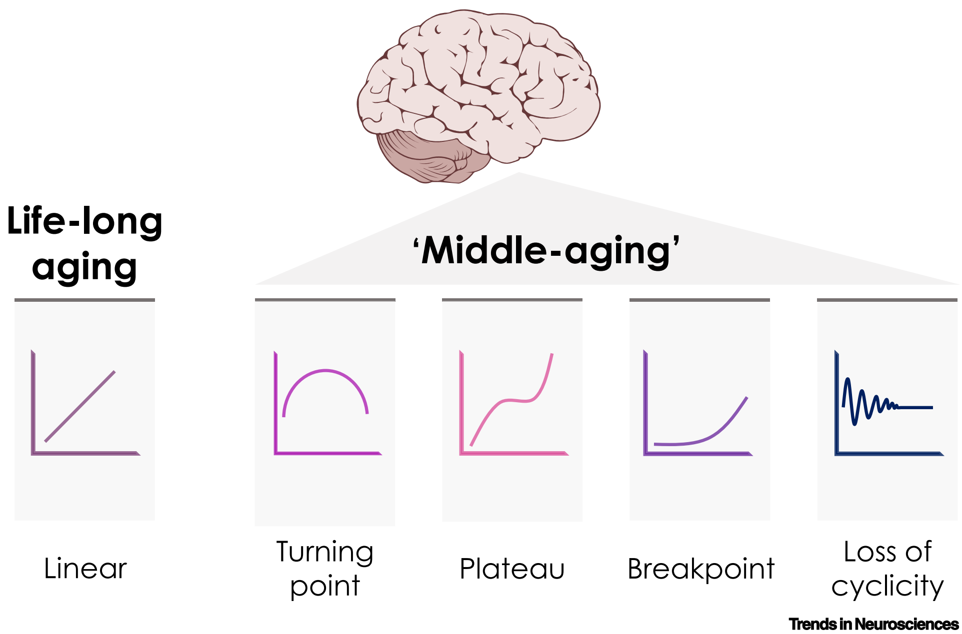

Figure 1. Different dynamics of aging across the lifespan and within the brain. Brain aging comprises many processes, operating at different biological levels, which can be leveraged as aging biomarkers (e.g., genes, proteins, metabolites, neural networks). Such processes can change according to different trajectories: some are linear and constant (indicating a gradual process that tracks chronological age and builds up over time), whereas others are non-linear and can change abruptly at different stages of life (exemplified here by turning points, plateaus, sudden breakpoints, and loss of cyclicity). These different dynamics could possibly be used to differentiate lifelong from life stage-specific aging processes (exemplified here by processes specific to middle age). Importantly, because different brain systems can age at different rates, they can be said to have different biological or functional ages at any given chronological age. This may have implications for the timing of interventions depending on which process they target. In this way, middle age could offer an intervention point to prevent accelerating brain aging and cognitive decline.

(e.g., retirement) are salient aspects of this stage of life [1]. In addition, interindividual differences in processing speed have been reported to amplify during this same transition ([41,42]; cf. [43]). Another interesting observation is that the benefits of 'practice effects' (i.e., repeated testing across study occasions) begin to disappear in longitudinal studies of episodic memory at around the age of 60 years [37,44]. This may prove clinically relevant in light of a recent finding that declining verbal memory practice effects in middle-aged and older adults are associated with the severity of cognitive impairment [45]. Thus, midlife could be a point where individual differences in future cognitive trajectories begin to emerge, such as those pertaining to early cognitive decline, given sufficiently sensitive measures. Indeed, a study [46] employing a test battery used in large population studies of cognitive aging (such as in [37]) found that carriage of the *ε4* allele of the apolipoprotein E (*APOE*) gene – a known risk factor for Alzheimer's disease (AD) – had a negative effect specifically on episodic memory in middle-aged but not younger adults. Accordingly, a larger study found measurable differences of *APOE ε4* dosage on longitudinal verbal memory trajectories over a decade in middle-aged individuals [47].

One aspect of episodic memory that may be particularly sensitive to aging is pattern separation (or 'mnemonic discrimination' when assessed behaviorally in humans). This function of the hippocampus allows for minimum interference storage and recollection of highly similar related memories. It is well known to decline with age, to be sensitive to subclinical cognitive impairment, and to associate with several AD biomarkers (reviewed in [48]). Although the decline in

Box 2. Studying middle age requires more than middle-aged subjects

Biological aging mechanisms, such as inflammation, feature both linear and non-linear processes [10,12,13]. A linear process tracks chronological age and could be said to apply equally across the lifespan, whereas a non-linear process might be especially relevant during some stages of life, especially when the progression accelerates, and this could indicate a shifting balance of opposing biological processes [63]. As a caveat, linear trajectories could be coupled to a phenotype (e.g., disease onset) via a threshold model, in which a gradual process cumulatively gives rise to a discrete outcome after it crosses some threshold, as has been proposed for the link between senescent cell burden and age-related disease incidence [133]. Thus, non-linear modeling of biological processes across the lifespan could prove useful for identifying life stage-specific – as opposed to lifelong – changes. For instance, a recent transcriptomic study of human skin found markedly more non-linear gene expression trajectories in females than males, and several genes displayed turning points at around age 50 years, which the authors speculate could be linked to the onset of menopause [120].

The choice of non-linear model can be decisive in estimating the age at which turning points occur. For instance, quadratic polynomials are highly sensitive to age range compared to local smoothing algorithms. Indeed, one study of human hippocampal volume found that raising the lower end of the age range shifted the fitted breakpoint by up to three decades, whereas a smoothing spline robustly placed it at around age 47 years [134]. Moreover, the risk of overfitting associated with the more parameterized spline model could be offset by the inclusion of a larger age range. By way of comparison, a previous review of lifespan neuroimaging studies found that the majority employed polynomial models and participants aged 55–85 years [135]. Although the utility of some non-linear models is currently limited by sample size [136], others such as generalized additive mixed models (GAMMs) are increasingly employed to capture complex lifespan trajectories robustly (e.g., [27]).

In 'omic' applications, the recent advent of differential expression sliding-window analysis (DE-SWAN) has allowed the identification of periods of change in the peripheral proteome [91–93,95,96] and epigenome [93,94] by conducting sequential tests along the study population age range at a given resolution. This stresses the need for both linear and non-linear modeling in aging research [36], and thus requires more than two age groups and, preferably, a representative sample of most of the organismal lifespan.

mnemonic discrimination appears to be roughly linear across the adult lifespan, binning indicates that the earliest detectable differences occur by early middle age and level off at around the age of 60 years ([49,50]; cf. [51]). One study [52] employed ultra-high field magnetic resonance imaging (MRI) to assess how the structure and activity of hippocampal subfields associate with mnemonic discrimination across the adult lifespan. Notably, it found non-linear turning points in the relationships between dentate gyrus volume, CA3 activity (the two regions most important for pattern separation), and mnemonic discrimination at between ~40 and ~60 years of age. These findings were argued to reflect a possible transition point during which neural changes associated with future cognitive decline begin to emerge. Based on recent work in mice [53], it is tempting to speculate that this could partly be due to dwindling levels of adult hippocampal neurogenesis (that is thought to be crucial for pattern separation) which has been reported to gradually decline during human adulthood [54]. However, it is important to note that the existence and possible functional relevance of adult hippocampal neurogenesis in humans as opposed to rodents is a matter of ongoing debate [55–59], and the exact mechanistic link to pattern separation (as understood mainly through rodent studies) remains to be fully elucidated ([60] for a discussion of the prevailing theories).

The midlife decline in memory may also extend to model organisms. A study involving thousands of mice and numerous behavioral tasks found that some aspects of hippocampus-dependent memory decline (e.g., spatial memory and contextual fear conditioning) were only seen in the 8–12-month-old group [61] (10–14 months would correspond to reproductive senescence and serves as the murine analog of human middle age [62]).

Taken together, some cognitive domains, especially episodic memory, seem to undergo accelerating change during middle age, which could reflect individual trajectories of emerging cognitive decline, likely underpinned by non-linear changes in the brain.

Structural and functional changes in the middle-aged brain

The structures of the brain continue to evolve throughout adulthood, and this is characterized by both linear and non-linear trajectories demarcated by turning points that are likely reflective of shifting balances between degenerative and developmental processes [63]. The hippocampus [63] and adjacent parahippocampal gyrus [64] are two such regions where volumetric turning points occur in humans during the fifth to sixth decades, and some hippocampal subfields shrink faster and earlier than others [65]. Importantly, this accelerating decline and its interindividual differences in magnitude are associated with overall cognitive status [65] and longitudinal episodic memory decline during the transition from middle to old age [66]. On a larger scale, cerebral white matter and ventricular volume both demonstrate non-linear decline and growth, respectively, in around the sixth decade of life, and individual variation in these structures is differentially associated with mild cognitive impairment and AD case–control status [27,63]. Indeed, most fasciculi (particularly the associational fibers) display inverted U-curve relationships across the lifespan, with turning points between the ages of 40 and 50 years [67]. These developmental dynamics likely underlie the mirrored trend in reaction time across the lifespan, which retards exponentially by the sixth decade [40]. Supporting this, transmission speed in frontoparietal long- and short-range fibers has been found to reach minimum conduction delays between the ages of 30 and 40 years, with the latter maturing more slowly [29]. Moreover, a small study established a tentative association (albeit influenced by cognitive reserve abilities) between cross-domain cognitive decline and deterioration of the cingulum bundle and right inferior fronto-occipital fasciculus in healthy 50- versus 40-year-old adults [42]. This coincides with the structural turning points of these fibers during the fifth decade [67].

In line with the lifespan trajectories of most white matter tracts, the structural and functional connectivity of some networks, such as the frontoparietal and default mode networks, demonstrates negative quadratic trends in which declines begin at around the age of 40 years [64]. These are paralleled by positive quadratic trends in other networks, such as the ventral attentional network, which have been argued to reflect compensatory mechanisms [64]. Interindividual variability in functional connectivity (i.e., correlated neural activity as assessed with fMRI) also reflects these trends of opposing positive and negative quadratic relationships for the attentional and frontoparietal networks, respectively, with turning points at around the sixth decade of life. Such variability is associated with both fluid intelligence and overt motor behavior [28]. In general, the average strength and number of functional connections appear to peak at ~50 years of age and then decline [28]. This decline during aging is further paralleled, in particular, by a loss of long-range functional connections [28]. Supporting this, the average anatomical distance of functional connections has been demonstrated to peak at around the same age [68]. Connectivity between brain areas allows them to form modules that together make up larger functionally segregated systems (e.g., the attentional and default mode networks). This is achieved by a balance of denser within-systems connections and sparser between-systems connections. With advancing age, correlations between systems have been found to increase in general, leading to lower system segregation and, presumably, loss of functional specialization (this has been termed 'dedifferentiation') [69]. Notably, loss of segregation among the higher associational systems (i.e., frontoparietal, attentional, default mode) appears to be non-linear with a breakpoint at around age 50 years (compared to a life-long linear trend for the sensorimotor systems), and such loss predicts declining episodic memory independently of age, with significant interindividual variability [69]. A more recent longitudinal study replicated this non-linear compared to linear loss of functional segregation in associative and sensorimotor systems, respectively, albeit it placed the non-linear breakpoint somewhat later, at age 58 years [70]. Analogously to the phenomenon of highly 'connected' individuals in society forming networks with each other (termed 'rich clubs'), the brain displays functional interconnectivity among its densest hub regions, and this organization appears to peak during middle age [68]. Indeed, a recent systematic review of 144 resting-state functional imaging studies proposed that the fourth and fifth decades of life may be a turning point in the organization of brain networks that is characterized by optimal efficiency, system segregation, and modularity, followed by accelerated decay of these properties [71]. Interestingly, a connectome approach to cognitive test domains (i.e., using similar graph theoretic metrics) found an analogous loss of efficiency and modularity in the transition from the fifth to the sixth decade [72].

Taken together, there is good evidence to suggest that the human brain undergoes non-linear structural and functional changes during middle age that have implications for cognitive functioning, and variations in these processes could account for individual trajectories in cognitive aging. Ultimately, these changes likely emerge from complex dynamics at the most fundamental levels of biology.

Cellular and molecular changes in the middle-aged brain

In the past decade, organismal aging has increasingly been studied using large-scale assays of biological features such as proteins, metabolites, (non-)coding RNAs, and DNA modifications, collectively referred to as 'omics' (reviewed in [73]). One foundational example involves the construction of so-called biological 'clocks' through machine learning algorithms that predict chronological age from such biological features, most notably using DNA methylation levels in blood samples [74]. Although the model employed is generally linear, clocks can 'tick' at different rates across organs [14] and follow different ticking 'functions' (in the mathematical sense) across the lifespan, some of which are non-linear [9]. An otherwise linear clock can contain sub-features that track chronological age non-linearly, sometimes in a breakpoint- or plateau-like manner [11]. Indeed, epigenetic clocks based on human brain tissue have been improved by taking non-linear relationships into account [75]. Importantly, epigenetic age has also been found to predict longitudinal cognitive functioning during middle age [76]. Thus, as captured by clocks, biological aging is an amalgamation of complex processes with different dynamics (not all of which are immediately damaging [77]), opening the possibility of identifying those that are especially relevant to a given period of life.

Relatedly, molecular processes – even those belonging to the same hallmarks of aging [78] – display both linear and non-linear trajectories across the lifespan [10,13]. Findings in support of this notion have emerged, for instance, from transcriptomic studies of human or rodent tissues employing a wide range of subject ages. In the human cortex and hippocampus there is some evidence to suggest that coexpression among genes undergoes exponential change between the sixth and seventh decades [79]. This involves increases in immune-related gene expression and decreases in the expression of some synaptic genes [79]. In particular, when gene expression heterogeneity among individuals is assessed across different datasets and brain regions, there appear to be non-linear changes during middle age and exponential increases emerging at around the age of 60 years in genes related to axon guidance and mTOR signaling (a highly conserved pathway consistently associated with longevity) [19]. A study of the human hippocampus similarly found an exponential increase in the expression heterogeneity of neurotransmission- and immune-related genes during the transition from the sixth to the seventh decade [20]. It is tempting to speculate that such divergence is indicative of differences in individual aging trajectories during middle age, with some people aging faster than others. Although the reasons for the gene expression variability remain to be fully explained, the rapidly increasing variance after the age of 60 years (that has also been detected as early as age 40 years) has been replicated using different methods [80] and technologies [19,20]. Interestingly, one study analyzing several datasets identified a gene expression profile composed of mTOR and mitochondrial pathways that was linearly associated (mainly negatively) with age in human brain, muscle, and skin, but this association was abruptly lost at around the age of 60 years [18]. This could be evidence of a non-linear breakpoint in the regulation of these two pathways during the transition from middle to older age, as has been suggested for mTOR in epidermal tissue [10].

Related to the aforementioned exponential increase in ventricular volume during middle age [27], a transcriptomic investigation of the choroid plexus, the main source of cerebrospinal fluid (CSF), in cognitively healthy participants found that the largest gene expression changes occurred during the sixth decade. These changes uniquely enriched for cell barrier-related pathways (e.g., downregulation of gap junction-related genes), whereas axon and neuron projection pathways were enriched among genes that continued to change (mainly increasing) well into the ninth decade of life [81]. Although participants younger than 50 years were not included, raising the possibility that the effects might have an earlier onset, these results are interesting in light of recent findings of clonal T cell recruitment to the CSF that is associated with neuroaxonal breakdown during both healthy and non-healthy aging from the sixth to the ninth decades of life [82]. Interestingly, this study found that the T cell receptor repertoire of cognitively impaired participants aged roughly 50–80 years was more similar to that of healthy participants aged 68+ years than those aged 54–67 years, implying accelerated biological aging as a feature of cognitive impairment. Relatedly, cognitive health in old age has been suggested to reflect the transcriptional aging trajectory of hippocampal formation structures during midlife [83]. A small proteomic study (i.e., analysis of the total protein population) of human hippocampal and occipital cortex synaptosomes revealed that some proteins showed breakpoint-like differential abundances between the ages 40–50 and 70+ years, and that these were enriched in the transforming growth factor β (TGF-β) pathway [21]. In the hippocampus specifically, this pathway was abruptly upregulated, which the authors speculate could represent a mechanism of age-related, regional synaptic vulnerability through proinflammatory microglial signaling. Crucially, mitochondrial, synaptic, and inflammatory protein expression (including microglial and TGF-β pathways) in the dorsolateral prefrontal cortex has been found to associate with individual longitudinal cognitive trajectories from late middle to old age in large independent cohorts [84], and these findings are in line with gene expression data from the same brain region in older AD case–control comparisons [85].

Supporting the notion that midlife is a period of accelerated change across species, a recent transcriptomic study of non-coding RNAs in mouse organs found that the strongest effects in the brain at 12 and 18 months were in the form of a miRNA coexpression turning point [12]. One non-coding transcript with such a trajectory in the brain (miR-29c-3p) was found to be sensitive to systemic rejuvenation through heterochronic parabiosis (i.e., the surgical joining of the circulatory systems of young and old animals) and was related to immunosenescence, possibly via TGF-β signaling. A similar investigation of coding RNA found non-linear trajectories in neuronal and synaptic (plateau), immune (breakpoint increase), and protein folding (breakpoint decrease) pathways during middle age in the mouse brain [13]. Although these effects may not necessarily translate to changes at the protein level [86], it is interesting to note that synaptic and proteostatic pathways in the mouse hippocampus have been found to become deregulated at the protein level (possibly through loss of circadian expression patterns) by 12 months [87,88] (cf. [89] for evidence of retained cyclicity in the human hippocampus).

In summary, the brain appears to undergo non-linear changes in pathways related to mTOR, mitochondria, synapses, and inflammation during middle age, and accelerating heterogeneity of gene expression may possibly mark the start of the seventh decade of life in humans. Intriguingly, not all changes appear to stem from the brain itself but may be driven by the periphery and other organs.

Possible peripheral regulators of brain 'middle-aging'

Factors in the systemic circulation – mainly proteins secreted by various organs – are understood to mediate brain aging in terms of cognition, plasticity, adult neurogenesis, and neuroinflammation (recently reviewed in [90]). Thus, by altering this peripheral profile (e.g., indirectly via exercise, or directly via heterochronic parabiosis), the biological time on the cellular clocks of the brain can be sped up or slowed down [17]. In light of this, it is particularly interesting to note that recent epigenomic (i.e., large-scale characterization of epigenetic modifications and chromatin structure) and proteomic studies have found that some of the largest peaks of change across the entire adult lifespan occur during middle age, as measured in plasma [91,92], peripheral blood monocytes [93,94], and muscle [93,95]. Many of these peaks involve inflammation- and immune-related pathways. Moreover, some plasma protein coexpression trajectories with turning points in middle age are enriched for nervous system-related pathways [96], and similar results have been found in muscle and monocyte proteomes and methylomes [93,95]. This suggests that the state of the systemic circulation during middle age may be predictive of individual cognitive aging trajectories. Indeed, a large longitudinal study demonstrated that the composite level of four inflammatory markers measured at baseline during middle age predicted the slope of cognitive decline up to 20 years later, and this effect was especially reliable for verbal episodic memory [35]. In follow-up studies of the same cohort, plasma proteomic analysis of samples taken at middle age revealed synaptic, proteostatic, and immune- and microglia-related proteins that were associated with incident dementia 25 years later, neuroimaging-derived phenotypes 5 years later (e.g., white matter hyperintensities), and CSF inflammatory load [33,34]. Interestingly, protein coexpression modules during midlife were differentially associated with near- compared to long-term dementia onset and were enriched for different biological pathways and tissues [33]. Dementia-associated proteins identified in midlife rarely overlapped with those identified in other older cohorts, suggesting that the peripheral signature of cognitive aging may evolve in a life stage-specific manner [33]. A similar cohort study of late middle-aged UK-born individuals (60–64 years) established associations between both cross-sectional and longitudinal measures of verbal memory/processing speed and plasma metabolites (mainly fatty acids and lipids), and these were mostly explained by childhood cognition and education [97]. Network-based analysis of the same metabolomic data revealed that coexpression modules (i.e., correlated subsets of metabolites) involving acylcarnitines and sphingolipids were associated with 5–9-year longitudinal memory and processing speed [98], and with cross-sectional hippocampal volume [99], respectively, which were robust to early-life factors, whereas other associations were largely explained by childhood factors. Some shifts in peripheral metabolite levels could be due to non-linear changes in human gut microbiota composition (i.e., the trillions of microbes inhabiting the gastrointestinal tract) during midlife [100], which may influence neuroinflammation and cognition, as suggested by work in rodents [101]. Thus, predictors of midlife cognitive trajectories appear to result both from ongoing (accelerating) aging processes and early-life determinants, and these may be differentially captured by longitudinal and cross-sectional study designs (Box 3).

In light of these findings, it is interesting to note that the biological age of peripheral organs could influence that of the brain. A recent study of the predominantly middle-aged UK Biobank cohort constructed linear age clocks (i.e., machine learning models that predicted chronological age from biological features) for the brain (using neuroimaging-derived phenotypes), for seven other organs (based on organ-specific clinical biomarkers), and for overall cognition [30]. Notably, higher brain age gaps (i.e., per individual differences between predicted and actual age, a proxy for biological age acceleration) were associated with higher organ age gaps when examined cross-sectionally, especially in the cardiovascular, pulmonary, and metabolic systems. Higher predicted brain and body ages also predicted an older cognitive age, and peripheral organs were found to accelerate the longitudinal age gaps in other organs. Finally, a similar approach by another group

Box 3. Cross-sectional and longitudinal middle age may be different

For many years the natural trajectory of cognitive decline with aging was subject to debate because cross-sectional designs estimate the beginning of decline during the third decade, whereas longitudinal designs put the start of the decline in the seventh decade [37]. Ultimately, this discrepancy could be explained by longitudinal retest effects (which are known to last for years [137]), cohort effects [138], and the fact that the two designs capture fundamentally different sources of variation [139]. Thus, the choice of design can bias estimates of age-related associations. Recently, similar discussions have arisen in lifespan neuroimaging research [122,140] where cross-sectional designs have been the norm both historically [135] and more recently [27]. Cross-sectional estimates of structural change appear to significantly underestimate actual longitudinal trajectories of decline, and these differences are partly attributable to technical confounds and participant lifestyle factors [122]. Moreover, cross-sectional and longitudinal estimates of brain age (i.e., chronological age predicted from neuroimaging-derived phenotypes using machine learning models) do not correlate (independently of statistical model and study population) and appear to reflect different influences, and the former (but not the latter) was specifically associated with early-life and genetic factors [140]. Mirroring the findings in psychology, differences with age do not equate to differences due to age-related change [71,140,141], emphasizing the need for longitudinal studies in both humans (e.g., [36]) and model organisms (e.g., [142]) to elucidate the causal drivers of brain aging. This parsing of biological age due to ongoing aging and congenital or early-life factors is reminiscent of Leonard Hayflick's distinction between aging (argued to occur due to increasing entropy) and longevity (suggested to be determined by genetic factors) [143]. Nevertheless, longitudinal biological age metrics also capture early-life and genetic influences [30,144,145], but, crucially, can adjust for these in estimates of ongoing change during adulthood [5,30].

Importantly, as biological age estimates (or 'aging clocks') are fundamentally statistical models that predict chronological age from biological features (e.g., DNA methylation, neuroimaging-derived phenotypes, protein levels), the aforementioned caveats would apply independently of the features being measured. Most aging clocks to date, however, are based on cross-sectional data (cf. [9] for a longitudinal example featuring non-linear trajectories), especially those relying on omic technologies (recently reviewed in [73]). To fully elucidate how biological processes during middle age shape future cognitive trajectories and brain health, longitudinal studies will be important.

found that the rate of aging of an individual based on 19 clinical body biomarkers at age 45 years was associated with white matter hyperintensity burden, lower hippocampal volume and processing speed, and poorer memory and sensorimotor functioning, even when adjusted for childhood intelligence quotient (IQ) and baseline health status two decades earlier [5,31].

In summary, middle age appears to be a period of significant change outside the brain, which may accelerate its aging through factors in the circulation (particularly those pertaining to inflammatory pathways) and, ultimately, shape individual cognitive trajectories.

Menopause – a defining feature of female middle age

A salient aspect of midlife not mentioned in our discussion thus far is reproductive senescence. In human females, this occurs naturally around the age of 50 years in the form of menopause, recently defined as the natural (or surgically induced) permanent loss of ovarian function, reproductive hormone production, and fertility [102]. Menopause has been found to accelerate epigenetic aging, as measured in female blood samples [103]. Indeed, its age of onset has a genetic architecture that mainly involves the DNA damage response [104] which could drive aging of the body, brain, and cognition through epigenomic information loss [105]. The causal influence of germ integrity on aging of the soma appears to be conserved throughout the kingdom of life and involves many pathways mentioned in previous sections, such as (m)TOR, TGF-β, and proteostasis [106].

In humans, menopause often entails subjective cognitive changes, although there is considerable interindividual variation [107]. The peri- to post-menopausal transition can be associated with non-linear declines across several cognitive domains and especially with impaired verbal episodic memory [108–110]. This transition – independently of age – is paralleled by accelerating hippocampal volume loss, brain glucose metabolism dysregulation, and white matter hyperintensity burden [110,111]. An earlier age of menopause is further associated with faster brain aging, as assessed via neuroimaging-derived phenotypes, such as total gray matter volume [112] and functional connectivity [30]. This is likely driven by complex hormonal dynamics. Interestingly, ovarian hormone fluctuations during the menstrual cycle were recently demonstrated to be associated with non-linear fluctuations in hippocampal subregion volume in a longitudinal study of young adult women [113]. Supporting the role of female reproductive senescence in driving complex brain changes, a study in female rats found that loss of cyclical estrogen was accompanied by differential regulation of TGF-β, interferon, and lipid metabolic pathways in the hippocampus (some of which were also found in middle-aged compared to younger adult human females) and possible immune infiltration of white matter [25]. This suggests that the menopausal transition could act as a breakpoint in several brain aging processes. However, it is important to note that the more traditional definition of menopause such as the cessation of menstrual cycles (i.e., time of final menses) does not apply to most species (including rodents), and is therefore not captured by common model organisms.

Although an in-depth review of the effects of menopause on the brain is beyond the scope of the current article, we note that this transition is likely to be a source of non-linear change in many of the studies mentioned above and itself deserves due consideration.

Concluding remarks and future perspectives

The trajectories of non-linear change in functional and structural brain connectivity, episodic memory, and gene and protein expression in the brain and systemic circulation during the fifth and sixth decades of human life suggest that middle age is a period of time during which unique processes shape future brain and cognitive health (Figure 2). Findings have largely emerged from studies on humans or model organisms that encompass most of the adult lifespan. This highlights the importance of including a wide subject age range when studying biological processes that display complex relationships with age because they cannot be sufficiently captured by simple two-group comparisons (e.g., young compared to old). The changes in functional brain networks and white matter integrity during middle age serve as good examples. In addition, it is becoming clear that longitudinal and cross-sectional studies sometimes capture different measures of age-related variation. In several studies discussed in this review, cross-sectional estimates of age effects often reflect early-life differences between subjects rather than ongoing change due to aging. Thus, to capture life stage-specific aging processes, the field would benefit from leveraging results from more studies with longitudinal designs.

Although we have discussed findings of relevance to cognitive aging, it is important to note that some associations are complex in nature and may represent compensatory responses rather than processes that drive aging *per se* [77]. Indeed, this has been the case with some plasma protein candidate markers of cognitive decline [33]. Ultimately, scientists will want to find novel therapeutic targets to mitigate unhealthy cognitive aging. Because targeting an age-related process that turns out to be compensatory could prove deleterious [77], it is important to identify which 'middle-aging' processes cause declining brain health (see Outstanding questions). A further layer of complexity arises from the observation that some biological aging processes appear to be partially reversible, such as the epigenetic age acceleration that results from exposure to different stressors in humans and rodents, but there is significant interindividual variation in the ability to reverse the acceleration [114]. If this variability extends to rejuvenation of the brain, predicting a response to intervention at an early stage may prove clinically useful.

There still remains a paucity of interventions against unhealthy cognitive aging. Physical exercise is often cited as a cost-effective and tractable alternative. Relevant to the discussion herein, exercise has been shown to result in the secretion of many different factors (e.g., proteins and metabolites) from body organs into the systemic circulation [115,116]. Some of these factors

Outstanding questions

Can middle age be (re)defined in a biology-informed manner?

Given the plethora of processes that change throughout the lifespan and affect future health outcomes, which are uniquely predictive during middle age?

Because the overall aging rate of any organ comprises many subprocesses, is there one or several (biological) middle ages of the brain, and, if so, can they be differentially targeted?

Which interventions are uniquely suitable to implement in middle age to mitigate future cognitive decline?

Which brain-related changes during middle age are mechanistically tied to cognitive decline, and which (if any) are neutral, adaptive, or represent changes in brain or cognitive reserve?

Which brain and cognitive changes during middle age are mediated by systemic drivers, and could some of these serve as molecular targets for intervention?

Several molecular processes show non-linear trends during middle age in cross-sectional designs, but which are due to accelerating, longitudinal aging, and which are artifacts of population effects (e.g., cohort effects) or early-life and congenital factors?

Are the brain aging trajectories of some people more amenable to intervention or reversal than others, and, if so, why?

Current geroscience strives to identify common and targetable pathways and mechanisms underpinning biological aging, and it is believed that such mechanisms overlap with those of prevalent age-related diseases, suggesting that healthy aging and diseases of aging lie on a continuum. If this is the case, how do the normal, non-linear ('middle-aging') processes discussed herein relate to the pathological processes that manifest in neurodegenerative processes that often emerge in middle age (e.g., β-amyloid plaque burden)?

Peripheral tissues have been found to display linear and non-linear gene

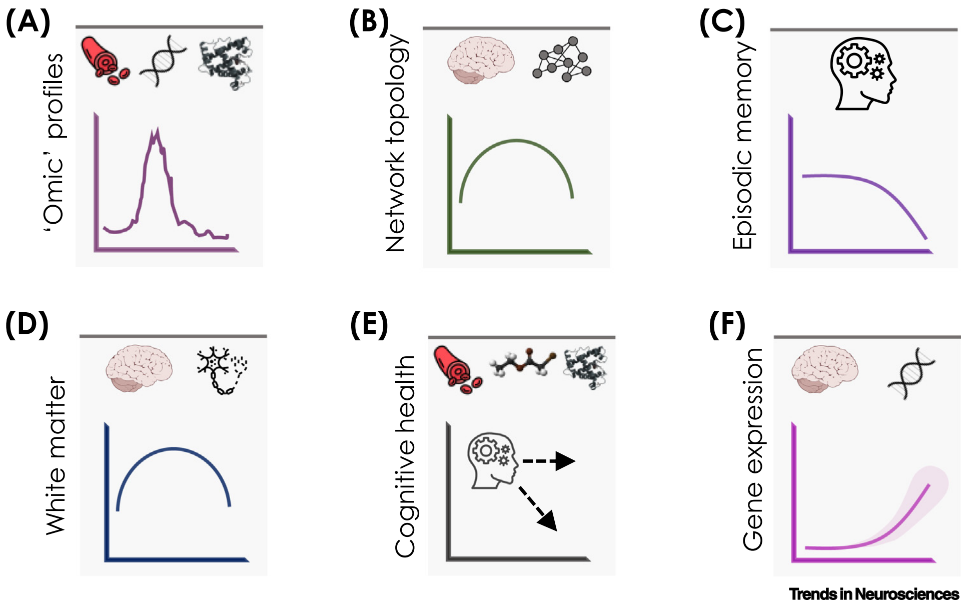

Figure 2. Changes in cognition, the brain, and periphery during middle age. Evidence from multiple levels of analysis suggests that middle age is associated with unique processes that can shape future cognitive aging trajectories. (A) The plasma proteome (including inflammatory pathways) and methylome of some peripheral tissues and cell types (e.g., muscle and blood monocytes) undergo waves of differential regulation, particularly at the start of the fifth and seventh decades of life (e.g., [91,93,94]). (B) The organization of functional brain modules (mainly involving associational networks) peaks and then begins to deteriorate (e.g., [68,69]). (C) Episodic memory shows an accelerating decline and possibly unstable individual longitudinal trajectories during the transition from middle to old age (e.g., [37,38,41])). (D) Structural (and possibly functional) connectivity, especially of associational fibers, reaches a peak and then starts to decline (e.g., [64,67]). (E) Proteins and metabolites in the circulation during middle age predict longitudinal cognitive health trajectories, sometimes up to two decades later (e.g., [32,35,97]). (F) Abrupt changes in gene expression and heterogeneity (involving inflammatory, synaptic, and longevity-related pathways) in the brain have been observed, especially toward the end of middle age (e.g., [18–20,79]). Icons were designed by SciDraw and Freepik.

have salutary effects on the aging brain, as seen in mice [117]. Interestingly, in both young [118] and middle-aged adults [119], brain plasticity-related pathways have been found to be enriched in the plasma protemic response to acute exercise, suggesting that the results may extend to humans. Thus, studying the intersection of aging- and exercise-related molecular processes could unveil new therapeutic targets.

Lastly, it is important to note that there are notable sex differences in aging-associated processes, such as a differential prevalence in dementia (reviewed in [120]). Molecular aging trajectories have also been found to differ [9]. For instance, the maintenance of cyclicity in brain gene expression [89] and the degree of non-linearity in gene expression trajectories during middle age [121] can be sexually dimorphic. These differences warrant the inclusion of participants across sexes in human studies and the analysis of age–sex interaction effects, as well as mechanistic studies of sex differences in aging using animal models.

The fifth and sixth decades of human life are emerging as being more than a mere interlude between adulthood and advanced age. Instead, non-linear trajectories at multiple levels of biology suggest that the brain may go through a phase of 'middle aging'. Ultimately, these changes could shape future cognitive aging. Therefore, renewed consideration should be given to this previously understudied period of life.

expression trajectories in a sex-dependent manner, especially during middle age. Are there aging processes of the brain that display similar sex-dependent dynamics?

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Declaration of interests

The authors declare no competing interests in relation to this work.

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