

Aging and Brain Rejuvenation as Systemic Events

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ABSTRACT

The effects of aging were traditionally thought to be immutable, particularly evident in the loss of plasticity and cognitive abilities occurring in the aged central nervous system (CNS). However, it is becoming increasingly apparent that extrinsic systemic manipulations such as exercise, caloric restriction, and changing blood composition by heterochronic parabiosis or young plasma administration can partially counteract this age-related loss of plasticity in the aged brain. In this review we discuss the process of aging and rejuvenation as systemic events. We summarize genetic studies that demonstrate a surprising level of malleability in organismal lifespan, and highlight the potential for systemic manipulations to functionally reverse the effects of aging in the CNS. Based on mounting evidence, we propose that rejuvenating effects of systemic manipulations are mediated in part by blood-borne 'pro-youthful' factors. Thus systemic manipulations promoting a younger blood composition provide effective strategies to rejuvenate the aged brain. As a consequence, we can now consider reactivating latent plasticity dormant in the aged CNS as a means to rejuvenate regenerative, synaptic and cognitive functions late in life, with potential implications even for extending lifespan.

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MAIN TEXT

Until recently, the aging process - the gradual detrimental effect of time on an organism that leads to death - was considered irreversible (Lopez-Otin *et al.* 2013). However, research over the last 30 years has challenged this assumption, providing compelling evidence that the aging process can be affected by several factors, including the genetic composition of the organism (Kenyon *et al.* 1993, Kenyon 2010), as well as the experiences the organism has with its environment (Alcedo & Kenyon 2004, Apfeld & Kenyon 1999, Jeong *et al.* 2012, Libert & Pletcher 2007). These findings indicate that aging is not a deterministic process, but is instead plastic, potentially availing itself to manipulation by means available to the fields of biology and medicine. The malleability of the aging process raises the exciting possibility that harnessing this plasticity may provide a means to slow or even reverse the aging process itself and rejuvenate physiological systems.

Aging at its core can be thought of as a systemic event. Indeed, the effects of aging do not occur in a targeted and isolated manner, but rather functionally alter tissues throughout the body (systemic aging), albeit at different rates (Lopez-Otin *et al.* 2013). With this in mind, individual tissues exhibit different levels of sensitivity and resilience to aging (Rando 2006). In particular, evidence suggests that the central nervous system (CNS) is especially vulnerable to the effects of aging (Mattson & Magnus 2006), experiencing a gradual loss in the ability to physically and functionally adapt to new experiences with age (Mahncke *et al.* 2006). Consequently, aging in the CNS results in decreased regenerative capacity for repair (Rando 2006) and impaired maintenance of synaptic and cognitive functions (Morrison & Baxter 2012). This detrimental influence of aging on the CNS is particularly alarming when considering the role of the CNS in regulating overall homeostasis (Alcedo *et al.* 2013a). Functionally, the CNS not only integrates sensory information from the external environment, but also responds to changes from within through communication with the systemic environment, collectively regulating important physiological processes including growth, metabolism, and reproduction (Alcedo *et al.* 2013a, Jeong *et al.* 2012, Libert & Pletcher 2007). More recently, the interactions of the CNS with the systemic environment have even been implicated in directly regulating organismal lifespan (Zhang *et al.* 2013). Thus, the CNS exhibits a unique duality not reflected in

other tissues, serving as both a vulnerable site to the effects of aging while also acting as a potential master regulator of systemic aging itself (Figure 1).

As with many aspects of the aging process, the loss of plasticity in the aged CNS was traditionally thought to be immutable (Merzenich *et al.* 2014). However, it is becoming increasingly evident that changes in the aged systemic environment through systemic manipulations such as exercise, caloric restriction (CR), and heterochronic parabiosis (in which blood composition is altered by connecting circulatory systems of young and aged animals) can partially counteract the age-related loss of plasticity in the aged CNS. Correspondingly, it is feasible to consider reactivating latent plasticity dormant in the aged CNS as a means to rejuvenate regenerative, synaptic and cognitive functions late in life, with potential implications for extending lifespan. In this review we will discuss the process of aging and rejuvenation as systemic events, drawing attention to the potential for systemic manipulations to functionally reverse the effects of aging in the CNS. First, we will highlight genetic studies that demonstrate a surprising level of malleability in organismal lifespan, the high degree of interplay between lifespan regulation and brain function, and how the brain may in turn be a key regulator of lifespan (Figure 1). Second, we will review evidence for rejuvenation, focusing on several systemic manipulations - exercise, CR, parabiosis and plasma administration (Figure 2) – and their ability to restore regenerative capacity throughout the body, as well as synaptic plasticity and cognitive function in the CNS (Table 1).

Plasticity of Aging: Lifespan as a Genetic Program

In the mid-1990's researchers began to seriously entertain the notion that a single gene could exert significant influence over organismal lifespan (Kenyon 2011, Kenyon *et al.* 1993). Since then, numerous studies have substantiated the notion that individual genes can determine how long an organism lives (Kenyon 2010), and opened up the possibility that a process as complex as aging could be manipulated at the molecular level. Though many more genes have been identified, the most well studied gene families shown to influence longevity, either through anti-aging or pro-aging effects, are the Insulin Receptor Signaling Pathway (van Heemst 2010, Fernandez & Torres-Aleman 2012), FoxO family of transcription factors (Webb & Brunet 2014, Kenyon 2010, Partridge & Bruning 2008), Sirtuins (Michan & Sinclair 2007, Baur *et al.* 2012), and the rapamycin target mTOR (Johnson *et al.* 2013). A significant part of our understanding of these genes and their influence on aging come from genetic studies in model organisms such as yeast, worms, and flies, and have more recently begun to be validated in higher organisms such as mice (Lopez-Otin *et al.* 2013), suggesting the conservation of these pathways for aging

across phylogeny. The effects of single gene manipulations on lifespan have previously been reviewed in greater detail (Johnson et al. 2013, van Heemst 2010, Michan & Sinclair 2007, Partridge & Bruning 2008), however, as several of these pathways will be mentioned throughout this review we will briefly describe them here.

Many of the genes shown to play a role in determining lifespan are involved in cellular processes that contribute to the aging of tissues, including oxidative stress resistance, glucose metabolism, and energy homeostasis, whose regulation ultimately lead to the survival or death of an organism (Lopez-Otin et al. 2013). The first molecular pathway regulating lifespan to be identified was the Insulin/IGF-1/FoxO pathway (Kenyon et al. 1993), an important signaling system that regulates energy homeostasis throughout the body (Fernandez & Torres-Aleman 2012). Whole organism mutations in a number of the insulin signaling pathway genes leading to reduced insulin signaling extend lifespan in worms, flies, and mice (Tatar *et al.* 2003, Kenyon 2005). In contrast, factors that act downstream of Insulin/IGF-1 signaling, such as the FoxO transcription factors, when up-regulated, act as pro-longevity signals that extend lifespan (Lin *et al.* 1997, Ogg *et al.* 1997) and also delay the onset and progression of age-related phenotypes (Partridge & Bruning 2008). Another important set of molecular mediators of longevity are the Sirtuins, a collection of histone deacetylase genes involved in oxidative metabolism and stress resistance (Baur et al. 2012, Michan & Sinclair 2007). Increased expression of several of the Sirtuins, such as Sirt1 (Sato *et al.* 2013) and Sirt6 (Kanfi *et al.* 2012), extend lifespan in model organisms from yeast to mice (Michan & Sinclair 2007, Kanfi et al. 2012, Sato et al. 2013, Kaeberlein *et al.* 1999, Sinclair *et al.* 1997, Tissenbaum & Guarente 2001). Lastly, another genetic pathway shown to regulate lifespan is the mTOR pathway. mTOR was originally discovered as a target of the immunosuppressant rapamycin (Heitman *et al.* 1991) and plays an important role in integrating multiple signals involved in cellular energy (Hay & Sonenberg 2004, Kapahi & Zid 2004). More recently, reduced activity of the mTOR pathway, often times via rapamycin administration, was also shown to promote longevity in model organisms (Harrison *et al.* 2009, Jia *et al.* 2004, Kaeberlein *et al.* 2005, Kapahi *et al.* 2004, Vellai *et al.* 2003). Collectively, the identification of such individual genes capable of modulating organismal lifespan first challenged long held dogma of aging as immutable, and introduced the notion that systemic aging could indeed be itself a plastic event.

Plasticity of Aging: Interplay Between Lifespan Regulation and Brain Function

Many of the pro-longevity signaling pathways have more recently also been shown to play important roles in higher-level brain function (Garelick & Kennedy 2011, Herskovits & Guarente

2014, Salih *et al.* 2012, Hoeffler & Klann 2010, Michan *et al.* 2010), providing evidence for a connection between the plasticity of aging and the potential plasticity of the aged CNS. For example, FoxO transcription factors have been implicated in regulating CNS function. In particular, FoxO6, which is most highly expressed in the hippocampus, was recently shown to play a role in memory consolidation in mice (Salih *et al.* 2012). The authors found that in the absence of FoxO6, mice were unable to consolidate the memory trace in contextual fear conditioning and novel object recognition tasks (Salih *et al.* 2012). Similarly, Sirt1 was recently also shown to be essential for normal cognitive function and synaptic plasticity in adult mice (Michan *et al.* 2010). Specifically, in the absence of Sirt1, mice displayed deficits in learning and memory processes such as immediate memory, classical conditioning, and spatial learning paradigms, while overexpression of Sirt1 enhanced these behaviors (Michan *et al.* 2010). Furthermore, these results were validated in an independent study that suggested beneficial effects of Sirt1 on cognition were mediated through a microRNA (miR-134)-dependent mechanism (Gao *et al.* 2010). Lastly, multiple lines of evidence have implicated mTOR signaling as an important molecular pathway for learning and memory (Halloran *et al.* 2012, Garelick & Kennedy 2011, Hoeffler & Klann 2010). Most of these studies showing the importance of mTOR signaling in memory are elicited via rapamycin treatment (Halloran *et al.* 2012, Hoeffler & Klann 2010). However, there are also genetic studies that validate the conclusion that mTOR signaling plays a role in learning and memory, though many genetic manipulations involve upstream or downstream targets of mTOR (Antion *et al.* 2008, Hoeffler & Klann 2010) as global genetic deletion of mTOR is embryonic lethal (Gangloff *et al.* 2004). Nonetheless, rapamycin treatment prevents memory consolidation across multiple brain regions including the hippocampus, auditory cortex, gustatory cortex, amygdala, and prefrontal cortex in rodents (Garelick & Kennedy 2011). Interestingly, in the FoxO (Salih *et al.* 2012) and Sirtuin (Michan *et al.* 2010, Gao *et al.* 2010) studies mentioned above the behavioral results were paralleled by changes in synaptic (long-term potentiation and long-term depression) or structural plasticity (spine density) in brain regions such as the hippocampus that are particularly vulnerable to the effects of aging. This suggests a possible parallel role for these signaling pathways in promoting longevity and potentially facilitating functional improvements in age-related cognitive dysfunction. This body of work not only demonstrated that longevity genes regulate critical CNS functions, but also offered the first evidence that just as organismal lifespan is malleable so too could CNS functions prove to be amenable to rejuvenation.

Plasticity of Aging: The Brain as a Central Regulator of Lifespan

The CNS is intimately associated with nearly every physiological system throughout the body (Alcedo et al. 2013a, Dietrich & Horvath 2013), placing it in a unique position to be influenced by and potentially influence systemic aging. Up to now, we have mainly considered how genetic manipulations of pro-longevity genes highlight plasticity in the aging process. However, considering aging is associated with a loss of function across multiple tissues, and the intimate association of the CNS with these tissues, we now address the mounting evidence for the CNS as a central regulator of systemic aging.

Some of the first evidence for the role of the CNS in controlling systemic aging came from cell-specific manipulations of longevity genes in model organisms such as worms, flies and mice. For example, restoring insulin signaling in neurons, but not muscle or intestine, was sufficient to increase the lifespan of worms (Wolkow *et al.* 2000). In flies, manipulation of signaling molecules that impact FoxO activity improved neuronal stress response capabilities to reactive oxygen species and extended overall lifespan (Lee *et al.* 2009). Additionally, mutations in genes that affect the ability of gustatory and olfactory sensory neurons to transmit systemic information about nutrient sources to the CNS, likely affecting metabolism, also regulated lifespan in worms and flies (Apfeld & Kenyon 1999, Alcedo & Kenyon 2004, Jeong et al. 2012, Libert & Pletcher 2007, Libert *et al.* 2007). There is now growing evidence that the same molecular pathways observed to regulate lifespan in the invertebrate CNS are also important regulators of aging in the mammalian CNS. In mice, reduction of brain insulin receptor substrate extended lifespan by 18% (Taguchi *et al.* 2007), an effect that is nearly identical to the changes in lifespan of mice that have heterozygous deletions of the insulin signaling substrate throughout the entire body (Taguchi et al. 2007, Bartke 2007). Additionally, mice with homozygous deletions of the insulin signaling substrate die in utero; however mice with homozygous deletion of this substrate specifically in the brain show a 14% increase in lifespan (Taguchi et al. 2007, Bartke 2007). These results demonstrate that, while complete loss of insulin signaling is detrimental to an organism, maintaining just one copy of this gene specifically in the CNS can significantly influence lifespan regulation promoting organismal longevity even in mammals (Bartke 2007). Together, these studies suggest that manipulation of longevity genes specifically in the CNS is sufficient to counteract the effects of aging at a systemic level.

More recently, aging research in mice has now begun to hone in on a particular region of the brain, the hypothalamus, as the potential CNS mediator of lifespan regulation (Tang & Cai 2013, Zhang et al. 2013). The hypothalamus is a region of the brain comprised of a diverse set of nuclei and is located between the thalamus and brain stem. Despite its small size, the

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hypothalamus receives input from nearly every tissue in the body, and by regulating endocrine signaling via hormones, plays a crucial role in many physiological processes including growth, metabolism and reproduction to name a few (Dietrich & Horvath 2013). Thus the hypothalamus serves as a well-established hub for communication between the CNS and the systemic environment. Indeed, it is the ability to both sense and influence tissues throughout the body that first indicated the hypothalamus as being well poised to regulate systemic aging. For example, studies have shown that brain overexpression of the pro-longevity gene Sirt1, specifically enriched in the hypothalamus, could extend lifespan in male and female mice (Satoh et al. 2013). Moreover, increased hypothalamic Sirt1 expression also improved age-related phenotypes including improvement of physical activity, oxygen consumption, sleep quality, and body temperature when compared to age-matched controls (Satoh et al. 2013). As with invertebrate studies, these data highlight the intimate interplay between pro-longevity mechanisms and CNS-mediated lifespan regulation. Independent studies have now also shown that the hypothalamus can directly regulate systemic aging itself through the immune-neuroendocrine axis (Zhang et al. 2013). Specifically, investigators showed that reducing inflammation in the hypothalamic immune cells (microglia) could change the hypothalamus response to the systemic environment extending lifespan and reducing aging-associated pathologies (Zhang et al. 2013). The authors also provide a potential mechanistic link between age-related changes in hypothalamic inflammation and systemic levels of gonadotropin-release hormone (GnRH). Additionally, manipulations of the immune-neuroendocrine axes also yielded significant enhancements in the CNS, including increased neurogenesis and improved cognition in aged mice (Zhang et al. 2013). While future studies remain necessary to better understand how age-related changes in the hypothalamus influence lifespan regulation, collectively these studies support the hypothesis that the CNS plays a central role in modulating systemic aging (Tang & Cai 2013, Gabuzda & Yankner 2013, Zhang et al. 2013, Bartke 2007, Satoh et al. 2013, Alcedo et al. 2013a).

Taken as a whole, studies in invertebrates and mammals highlight the malleability of the aging process and demonstrate the involvement of the CNS in regulating lifespan (Alcedo *et al.* 2013b, Alcedo et al. 2013a, Boulianne 2001, Zhang et al. 2013), raising the possibility that influencing the communication between the CNS and the systemic environment could prove one effective strategy for counteracting the effects of aging (Tang & Cai 2013).

Reversal of Aging: Systemic Manipulations as Mediators of Rejuvenation

Manipulating individual genes within the CNS may represent one approach with potential to counteract the effects of aging (Bowers *et al.* 2011). However, targeted manipulations of genes, specifically within specialized populations of neurons in regions such as the hypothalamus, prove challenging at this point (Kay 2011, Wanisch *et al.* 2013). While reversal of aging by the CNS appears distant, alternative strategies to rejuvenate aged tissues through a more systemic approach may prove equally effective in counteracting the aging process. In this manner, broad changes in the aged systemic environment, rather than a central regulator, may provide the means for rejuvenation. Indeed, systemic manipulations such as exercise, CR and changes in blood composition by heterochronic parabiosis or young plasma administration (FIGURE 2) have already yielded much promise in their ability to combat signs of aging in both peripheral tissues and the CNS. Specifically, to date, these different systemic manipulations have been shown to ameliorate impairments in the regenerative capacity of aged tissues, as well as synaptic plasticity and even cognitive functions in the aged CNS. While exercise, CR, and changes in old blood composition have not been shown to ameliorate all of the same set of age-related impairments or to the same degree, evidence that a systemic approach to rejuvenation is plausible continues to mount. It should be noted, that the field of rejuvenation research remains in its infancy and questions such how systemic manipulations can rewind the aging clock remain poorly understood.

Reversal of Aging: Rejuvenation of Regeneration by Systemic Manipulations

One feature of aging that appears to be common among tissues is the loss of regenerative capacity (Jones & Rando 2011, Rando 2006, Conboy & Rando 2005). Critical for regeneration throughout life is the maintenance of endogenous tissue specific adult stem/progenitor cells, which have the ability to self renew and produce new cells in adult tissue (Conboy & Rando 2005, Rando 2006, Weissman 2000, Jones & Rando 2011). With age comes functional failure of adult stem/progenitor cells, serving as one potential contributor to the regenerative decline of both peripheral tissues and the CNS during aging (Jones & Rando 2011, Conboy & Rando 2005). For example, the decline in muscle regeneration is common in the elderly, and likely increases the chance of accidents and the ability to perform manual tasks (Conboy & Rando 2005, Grounds 1998). Studies suggest that this decline in skeletal muscle function and mass with age is mediated by the inability of muscle satellite cells (progenitors) to regenerate muscle fibers (Conboy & Rando 2005). Similarly, in the blood, dysfunctional regeneration due to aging of hematopoietic stem cells has been linked to inefficient immune responses, anemia, and

leukemia in the elderly (Ergen & Goodell 2010, Corey *et al.* 2007). Interestingly, the adult CNS was traditionally thought to be devoid of significant regenerative capacity. However, we now know that specific brain regions including the subventricular zone (SVZ) lining the lateral ventricle and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus harbor adult neural stem/progenitor cells capable of generating neurons (neurogenesis) (Lee *et al.* 2012, Ming & Song 2011, Eriksson *et al.* 1998). Consistent with peripheral tissues, the levels of adult neurogenesis also decline with age (Lee *et al.* 2012, Kuhn *et al.* 1996, Bondolfi *et al.* 2004). While adult neurogenesis has been shown to regulate cognitive functions in young adults (Clelland *et al.* 2009, Dupret *et al.* 2007, Nakashiba *et al.* 2012) data still remain inconclusive and predominantly correlative with regards to decreased neurogenesis and cognitive dysfunction in the elderly (Lee *et al.* 2012). Notwithstanding, this does not preclude the possibility that increasing adult neurogenesis in the aged brain may facilitate cognitive improvements in the elderly (Lee *et al.* 2012). Correspondingly, strategies that enhance regeneration in both peripheral tissues and the CNS may prove efficacious at counteracting the deleterious effects of aging on tissue function as they arise with age (Conboy & Rando 2012). Here we will review present evidence for rejuvenation of regeneration by systemic manipulations that include exercise, CR and heterochronic parabiosis (Table 1).

Exercise: Physical exercise increases blood delivery to most tissues and leads to changes in the systemic environment (Cotman *et al.* 2007, Fabel *et al.* 2003, Trejo *et al.* 2001). Interestingly, numerous studies have documented rejuvenating effects of exercise on the functional and regenerative capacity of peripheral tissues and CNS in animal models (Baker *et al.* 2011, Shefer *et al.* 2010, Valero *et al.* 2012, van Praag *et al.* 1999a, van Praag *et al.* 1999b, van Praag *et al.* 2005). Moreover, exercise has even been associated with a reduced incidence of developing classical age-related diseases including cardiovascular disease, type II diabetes, osteoporosis, macular degeneration, and dementia (Larson *et al.* 2006, Lawson *et al.* 2014, Mora *et al.* 2007, Warburton *et al.* 2006). Considering loss of tissue regeneration during aging is thought to contribute to decreased tissue function (Jones & Rando 2011, Conboy & Rando 2012), it is consistent with the concept of rejuvenation that the beneficial effects of exercise extend to enhancements in adult stem/progenitor cells in old age. Outside the CNS, exercise can promote hematopoiesis (regeneration of blood cells) in the aging systemic environment (Baker *et al.* 2011), and increase the proliferative capacity of aged skeletal muscle stem cells, including satellite (Shefer *et al.* 2010) and mesenchymal stem cells (Valero *et al.* 2012). Recent studies have shown the effects of exercise on molecular and cellular processes that play major roles in stem cell function both in *in vitro* and *in vivo*. For example, exercise induces autophagy

(clearance of cellular debris) (He *et al.* 2012), which protects hematopoietic stem cells from metabolic stress and contributes to their lifelong maintenance (Warr *et al.* 2013). Exercise is also associated with increased telomere length in humans (LaRocca *et al.* 2010), which may aid in counteracting the effects of telomere shortening on replicative senescence (Allsopp & Weissman 2002, Flores *et al.* 2005). On a molecular level, exercise leads to regulation of telomere-associated genes and microRNA expression (Chilton *et al.* 2014), both important for stem cell self renewal and differentiation (Gangaraju & Lin 2009, Lee *et al.* 1998, Jaskelioff *et al.* 2011).

The beneficial effects of exercise extend beyond peripheral tissues to also include the brain. In particular, increased running in aged mice has been shown to enhance neural progenitor proliferation and neurogenesis (Kronenberg *et al.* 2006, Marlatt *et al.* 2012, van Praag *et al.* 2005, Wu *et al.* 2008) to a level comparable to that observed in young animals. Because of the blood brain barrier, it was traditionally thought that the beneficial effects of exercise on the CNS were not orchestrated through systemic changes in the periphery. However, recent studies suggest that the effects of exercise are, in part, mediated by changes in the systemic environment. Investigations looking at magnetic resonance imaging (MRI) measurements of cerebral blood volume (CBV) in the hippocampus have demonstrated that exercise selectively increased the CBV of the dentate gyrus, correlating with postmortem increase in neurogenesis (Pereira *et al.* 2007). From a molecular perspective, elevated systemic levels of circulating growth factors such as vascular endothelial growth factor (VEGF) and insulin-like growth factor 1 (IGF-1) in blood elicited by increased exercise have been shown to mediate, in part, enhancements in neurogenesis (Fabel *et al.* 2003, Trejo *et al.* 2001). Coincidentally, circulating levels of IGF-1 decrease with age and the restoration to levels resembling a younger systemic environment up-regulate neurogenesis and improve learning and memory (Darnaudery *et al.* 2006, Lichtenwalner *et al.* 2001).

Together, these studies show that a systemic manipulation such as exercise can rejuvenate adult stem cell function across tissues. Additionally, it indicates that targeting the systemic environment could prove to be an effective strategy to reverse the functional impairments of aging on the aged CNS.

Caloric Restriction: Another systemic manipulation shown to counteract the age-induced effects on tissue regeneration is CR, a reduction of 20-40% of caloric intake without malnutrition. The ability of CR to counteract aging was initially characterized as an extension of studies investigating the pro-longevity effects of CR on lifespan, a phenomenon conserved across phylogeny (Libert & Guarente 2013, Fontana *et al.* 2010). This effect likely results from

increasing glucose metabolism, reducing oxidative stress and the ability of cells to counteract DNA damage, as well as influencing aspects of the aging immune and neuroendocrine system (Fusco & Pani 2013). More recently CR has been shown to rejuvenate tissue regeneration in aged organisms (Mazzoccoli *et al.* 2014), similar to the effects of exercise. A number of studies have shown rejuvenating effects of CR on the decline of hematopoietic stem cell function (Ertl *et al.* 2008, Grymula *et al.* 2014, Chambers *et al.* 2007). Rejuvenation of regeneration was also observed in skeletal muscle, in which short-term CR in aged animals increased muscle stem cell availability and activity when compared to ad libitum fed mice (Cerletti *et al.* 2012). Additionally, there are also beneficial effects of CR on intestinal stem cells. Specifically, a 35% increase in intestinal Olfm4-positive progenitor cells was observed in mice under CR compared to ad libitum fed controls, indicating that CR promotes intestinal stem cell self renewal and preservation (Yilmaz *et al.* 2012).

The effects of both short-term and long-term CR on rejuvenation of regeneration are also found in the CNS. Early studies observed an increase in proliferating cells in the dentate gyrus after a 30% reduction of caloric intake for three months in rats (Lee *et al.* 2000) and for two weeks in mice (Lee *et al.* 2002). However, different from peripheral tissues, this effect of CR on increased cell number in the brain resulted from a reduction in cell death, rather than an increase in cell proliferation (Lee *et al.* 2000, Lee *et al.* 2002). Additionally, only BrdU, a marker of cell proliferation, was quantified making it difficult to conclude that this increase in BrdU-positive cells translates into increased levels of neurogenesis (Lee *et al.* 2000, Lee *et al.* 2002). Similar effects were also observed when mice were calorie restricted over an extended duration of 3 to 11 months, although in this study the authors found that CR led to an increase in glial cells and not neurons (Bondolfi *et al.* 2004). More recently, prolonged exposure of mice to CR (40% decrease in caloric intake maintained for 10-12 months) was shown to reduce age-related decrease in neural progenitor cell divisions in the aging brain (Park *et al.* 2013). As a whole, because different experimental paradigms of CR were used and the level of detail with which proliferating cells were characterized varied, the effect of CR on mammalian adult neurogenesis is difficult to ascertain with clarity. Future studies are therefore needed to better understand the effect CR plays in counteracting the age-related decline in adult neurogenesis. Nevertheless, CR illustrates that an additional systemic manipulation independent of exercise can also affect regenerative capacity in the aged brain.

Heterochronic Parabiosis: Lastly, rejuvenation of tissue regeneration in aging organisms has also been observed after heterochronic parabiosis. First introduced in 1864 by Dr. Paul Bert, parabiosis is a surgical procedure by which two animals are physically connected.

The procedure and its historical context have previously been described in greater detail (Conboy *et al.* 2013, Eggel & Wyss-Coray 2014). Of particular interest is the effects of the blood of young animals on the physiology and behavior of older animals. Through the use of the heterochronic parabiosis model, it has now been shown that exposure to young blood can rejuvenate the regenerative capacity of peripheral tissues and CNS in aged animals.

Several studies have shown that aged mice exposed to a young systemic environment exhibit reduced signs of biological aging in cardiovascular, skeletal, and digestive systems. In skeletal muscle, old heterochronic parabionts exposed to young blood exhibited increased muscle progenitor cell activity leading to enhanced regeneration and reduced fibrotic responses compared to old isochronic parabionts (Brack *et al.* 2007, Conboy *et al.* 2005). Liver tissue isolated from old heterochronic parabionts was also found to have more youthful levels of hepatocyte rejuvenation (Conboy *et al.* 2005). Furthermore, the rejuvenating effects of young blood have also been observed on both cardiovascular (Loffredo *et al.* 2013) and metabolic (Salpeter *et al.* 2013) systems in aging mice, although in these studies the effect of young blood on stem cells function was not investigated. In particular, young blood can reverse age-related cardiac hypertrophy in old heterochronic parabionts (Loffredo *et al.* 2013) and reverse the age-related decline in pancreatic beta cell replication thought to contribute to the development of type II diabetes (Salpeter *et al.* 2013). Together, these studies show that changing the composition of the aged systemic environment to a more youthful state can reverse the regenerative and functional decline in aged peripheral tissues. Indeed, these studies point to the existence of 'pro-youthful' factors in young blood that have begun to be identified in recent studies. For instance, restoring systemic levels of the growth differentiation factor 11 (GDF11), a member of the transforming growth factor beta (TGF β) superfamily (McPherron *et al.* 2009), to a more youthful state reversed age-related skeletal muscle and cardiovascular impairments in mice (Loffredo *et al.* 2013, Sinha *et al.* 2014). The authors found that daily administration of recombinant GDF11 into aged mice increased muscle satellite cell frequency and function, as well as improved muscle function (Sinha *et al.* 2014). Similarly, treatment of old mice with recombinant GDF11 reversed age-related cardiac hypertrophy (Loffredo *et al.* 2013). Another study also found that increasing levels of oxytocin, a circulating hormone, in aged mice enhanced muscle regeneration by increasing muscle stem cell activation and proliferation (Elabd *et al.* 2014).

The rejuvenating effects of young blood have now also been observed in the aged CNS of old mice. In a mouse model of demyelination, exposure of old mice to a youthful systemic environment increases myelination in the spinal cord of old heterochronic parabionts by

recruiting young peripheral monocytes and promoting differentiation of oligodendrocyte progenitor cells (Ruckh *et al.* 2012). These findings are of particular significance given that nerve demyelination occurring in the elderly functional impairs muscle strength and sensory discrimination (Verdu *et al.* 2000) while contributing to axonal degeneration (Edgar & Nave 2009). In the hippocampus, old heterochronic parabionts exposed to a young systemic environment exhibited increased neurogenesis in the dentate gyrus, indicated by increased BrdU-positive proliferating cells, Sox2-positive progenitors, and Doublecortin-positive newly born neurons (Villeda *et al.* 2011). More recently, an independent study also corroborated the rejuvenating effects of young blood on adult neurogenesis in the SVZ of old heterochronic parabionts (Katsimpardi *et al.* 2014). Specifically, they found that young blood increased Ki67-positive proliferating cells in the SVZ, enhanced olfactory neurogenesis, and facilitated vasculature remodeling in neurogenic regions of old mice. Interestingly, the authors found that recombinant GDF11 administration could, in part, mimic the beneficial effects of young blood on neurogenesis and vasculature remodeling (Katsimpardi *et al.* 2014). Consistent with other functions of the TGF β superfamily, GDF11 appears to have pleiotropic qualities. Indeed, during development, GDF11 participates in patterning of the CNS and has been found to inhibit embryonic neurogenesis (Hastings & Gould 2003, Wu *et al.* 2003, Wu & Hill 2009). Surprisingly, in the context of aging it seems to promote plasticity of the CNS (Katsimpardi *et al.* 2014). Identification of this one factor, GDF11, is an exciting finding, however the effects of young blood on rejuvenation of regenerative capacity are not fully recapitulated (Laviano 2014), suggesting the existence of multiple 'pro-youthful' factors. Given the rejuvenating effects of young blood have now been observed in neurogenic zones across multiple brain regions this supports the possibility of global rejuvenating effects that may extend throughout the aged brain.

Reversal of Aging: Rejuvenation of Cognitive Functions by Systemic Manipulations

Thus far we have focused on the ability of systemic manipulations to rejuvenate regenerative capacity, highlighting the recent body of work in the CNS focused on adult neurogenesis. However, functionally the relevance of the age-related decline in adult neurogenesis on cognitive impairments in the aged brain remains obfuscated (Lee *et al.* 2012). Given the hardship age-related cognitive impairments cause on the elderly, it is critical to consider the significant impact systemic manipulations could have if rejuvenating effects extend to higher order cognitive functions. Currently, there are now a number of studies demonstrating a potential role for exercise, CR, and young blood plasma administration in ameliorating age-

related cognitive impairments in learning and memory. However, at present the exact effects of exercise and CR on cognitive function remain ambiguous.

Exercise: Numerous studies have observed positive effects of exercise on cognition in both animal models and humans (Hawkins *et al.* 1992, Kramer *et al.* 1999, Kumar *et al.* 2012, Marlatt *et al.* 2012, O'Callaghan *et al.* 2009, Radak *et al.* 2001, Snigdha *et al.* 2014, van Praag *et al.* 2005, van Uffelen *et al.* 2008a). In aged mice exposure to wheel running for one month resulted in better learning acquisition and memory retention in the Morris water maze task (van Praag *et al.* 2005). When aged rats exercised for 10-12 weeks they were observed to perform better on cued and object recognition tasks when compared to non-exercise aged matched controls (Kumar *et al.* 2012). Lastly, regular swimming exercise in aged rats was also associated with better performance on both short and long-term memory measured using the passive avoidance test (Radak *et al.* 2001). Consistent with findings in rodents, canines exposed to short and long-term exercise paradigms demonstrated enhanced cognitive function in discrimination and spatial learning and memory tasks, as well as object location and reversal learning (Snigdha *et al.* 2014). In humans, one study reported that 60-85 year old individuals who underwent 10-weeks of exercise performed better in a dual-task performance task compared to the non-exercise control group (Hawkins *et al.* 1992). Furthermore, a second study also showed that aerobic exercise for six months enhanced executive function in 60-75 year old adults (Kramer *et al.* 1999). It should be noted, however, that some contradictory studies argue against the beneficial effects of exercise, reporting that increased exercise does not modify spatial memory (Barnes *et al.* 1991) or ameliorate spatial memory deficits (Kumar *et al.* 2012) in aged rats. In older human adults with mild cognitive impairments increased exercise was also not observed to improve age-related memory and attention impairments (van Uffelen *et al.* 2008a, van Uffelen *et al.* 2008b).

Caloric Restriction: Several studies in animal models have observed beneficial effects of various forms of CR on a variety of cognitive functions such as motor learning and hippocampal-dependent memory (Ma *et al.* 2014, Talhati *et al.* 2014, Pitsikas & Algeri 1992, Dal-Pan *et al.* 2011, Fontan-Lozano *et al.* 2007, Witte *et al.* 2009). During long-term CR applied for 30 weeks adult mice were shown to have improved spatial learning and memory in the Morris water maze task (Ma *et al.* 2014). In a second study, short-term CR was also shown to enhance memory persistence in a discriminative avoidance task in young adult animals when applied before training and in aged animals when applied before testing (Talhati *et al.* 2014), indicating age-dependent differences in the effectiveness of CR. In primates, chronic CR also resulted in improved working memory (Dal-Pan *et al.* 2011). In humans, one study found that

CR improved verbal memory scores by 20% in 60-year-old males and females (Witte et al. 2009). Although a majority of studies have observed beneficial effects of CR on cognitive function, a number of studies have put into question the efficacy and extent to which CR can ameliorate age-related cognitive impairments (Beatty *et al.* 1987, Bond *et al.* 1989, Yanai *et al.* 2004, Martin *et al.* 2007). For instance, long-term CR in aged rats resulted in deficits in spatial discrimination tasks compared to ad libitum age-matched control groups (Yanai et al. 2004). Similarly, short-term CR was shown to impair memory consolidation and exert no effect on memory retrieval in aged mice (Talhati *et al.* 2014). In humans, while CR improved verbal memory (Witte et al. 2009), no beneficial effects were detected for other types of age-related memory impairments. Additionally, a randomized controlled study initiated by the National Institute of Aging found that after 6 months of CR there were no obvious effects on verbal memory scores, visual retention, or attention when compared to control groups (Martin et al. 2007). However, the age group of participants in this study had a range of 25-45 years of age, raising the question of whether an effect would be present in older humans.

Despite the contradictory results described above, as a whole, the literature supports the notion that systemic manipulations such as exercise, and to some extent CR, elicit beneficial effects on cognitive impairments (Kramer & Erickson 2007, Hillman *et al.* 2008). Moreover, the disparities between studies investigating the effects of exercise and CR on ameliorating age-related cognitive impairments, to some degree, may likely reflect experimental design differences, including treatment duration, age of initiation, treatment regiment, and experimental group composition such as gender and weight (Kramer & Erickson 2007, Witte et al. 2009, van Uffelen et al. 2008a). Therefore, future studies and comparisons should strive to standardize these parameters in order to better understand the contribution that these systemic manipulations may have on preventing age-related cognitive impairments.

Heterochronic Parabiosis and Young Plasma Administration: Excitingly, recent research has begun to investigate the beneficial effects of changing old blood composition to a more youthful systemic environment on age-related impairments in cognition. Old mice administered young plasma over a period of less than a month demonstrated improvements in hippocampal-dependent learning and memory in water maze and contextual fear conditioning tasks compared with old mice receiving age-matched old plasma (Villeda *et al.* 2014). Another study found that old mice exposed to a youthful systemic environment by heterochronic parabiosis also showed enhancements in their ability to discriminate odors (Katsimpardi et al. 2014). Whether increased discriminability found in this study reflects an increased sensitivity of peripheral sensory receptors, or an improvement of more cognitive faculties such as learning

and memory, remains to be determined. Nonetheless, together, these studies show that exposure of an old mouse to a young systemic environment can reverse behavioral and cognitive impairments. These studies also demonstrate the potential for future therapeutic avenues aimed at restoring CNS functions by promoting a more youthful systemic environment. It is exciting to postulate that perhaps similar to heterochronic parabiosis, changes in the systemic environment occurring as a result of exercise or CR may actually be changing the composition of old blood back towards a more youthful state. Indeed, it would be interesting to compare the blood composition between old animals undergoing these different systemic manipulations. Altogether, however, these data all suggest that maintaining high systemic levels of 'pro-youthful' factors in the periphery has the capacity to counteract age-related functional impairments in the aged CNS.

At a mechanistic level, evidence now exists that beneficial effects of young blood on cognitive function may be mediated, in part, independent of stem cell rejuvenation by enhancements in alternative forms of brain plasticity. Indeed, three correlates of synaptic plasticity were increased in old heterochronic parabionts: synaptic strength, dendritic spine density and plasticity-related gene expression (Villeda et al. 2014). Extracellular electrophysiological recordings in hippocampal slices of old heterochronic parabionts revealed enhanced long-term potentiation, a process involving long term strengthening between neurons (Bliss & Collingridge 1993), compared to old isochronic parabionts (Villeda et al. 2014). Additionally, mature hippocampal granule cell neurons also exhibited structural enhancements in dendritic spine density, a proposed neuronal locus of plasticity (Sala & Segal 2014), after exposure to a young systemic environment (Villeda et al. 2014). In line with these results, microarray analysis of hippocampal tissue from old heterochronic parabionts identified expression profiles indicative of enhanced synaptic plasticity. In particular, activation of the cyclic AMP response element binding protein (Creb) signaling pathway was observed to mediate the rejuvenating effects of young blood on mature neurons in the hippocampus of heterochronic parabionts (Villeda et al. 2014). Moreover, Creb activation specifically in mature hippocampal neurons was also demonstrated to mediate cognitive rejuvenation after young plasma administration (Villeda et al. 2014). Notably, findings observed with heterochronic parabiosis and young plasma administration are consistent with previous reports in which synaptic plasticity and expression of plasticity-related molecules are also enhanced in the aged brain by other systemic manipulations including exercise and CR (Eckles-Smith *et al.* 2000, Fontan-Lozano et al. 2007, Marlatt et al. 2012, O'Callaghan et al. 2009). Interestingly, the structural and molecular rejuvenating effects of young blood by heterochronic parabiosis and

young plasma administration were primarily observed in the dentate gyrus and not CA1 region of the hippocampus, suggesting that 'pro-youthful' factors may elicit molecular changes selectively within the CNS regions susceptible to the effects of aging (Villeda et al. 2014).

Taken together, these studies are the first to highlight the rejuvenating effect a young systemic environment exerts on higher-level cognitive function in the old brain (Katsimpardi et al. 2014, Villeda et al. 2014). Excitingly, observing rejuvenation of plasticity at a molecular, structural, and functional level now opens the possibility that global age-related impairments in plasticity can be restored throughout the aging brain. However, whether enhancements of young blood extend beyond neurogenic regions such as the hippocampus to other areas of the brain lacking regenerative capacity such as the neocortex is unknown. Thus, future studies are now needed to determine the extent to which age-related functional impairments throughout the CNS can be reversed.

CONCLUSION

In this review, we have discussed how a growing body of work focusing on genetic and even CNS regulation of lifespan has demonstrated the plasticity of aging and its amenability to modulation (Figure 1). This work has raised the exciting possibility that we can tap into this latent plasticity as a means to counteract or even reverse signs of systemic aging. As our understanding of this plasticity grows, the more we can expect it to guide the development of strategies aimed at reversing age-related impairments. One such strategy that has emerged from our understanding of the plasticity of aging is the possibility for rejuvenation through external manipulations that alter the systemic environment (Figure 2). Here, we have highlighted the effects of exercise, CR, heterochronic parabiosis and young plasma administration in rejuvenating multiple forms of plasticity throughout the aging body, with particular emphasis on the CNS (Table 1).

To date, the rejuvenating effects of exercise and heterochronic parabiosis have been attributed, at least in part, to changes in the composition of the systemic environment, suggesting the presence of 'pro-youthful' factors. Indeed, elevated systemic levels of VEGF and IGF-1 during exercise mediate enhancements in neurogenesis (Fabel et al. 2003, Trejo et al. 2001). Moreover, restoring systemic levels of IGF-1 to more youthful levels in aged animals also enhances neurogenesis and improves learning and memory (Darnaudery et al. 2006, Lichtenwalner et al. 2001). Similarly, heterochronic parabiosis studies have demonstrated that restoring systemic levels of GDF11 to resemble a more youthful systemic environment also elicits enhancements in neurogenesis and neurovasculature remodeling in aged mice

(Katsimpardi et al. 2014). It is remarkable that 'pro-youthful' factors, such as GDF11, are being identified (Katsimpardi et al. 2014, Loffredo et al. 2013, Sinha et al. 2014), however it should be noted that at present the effects of young blood have not been fully recapitulated, pointing to the existence of additional 'pro-youthful' factors (Laviano 2014). While it is critical that future studies continue to explore the rejuvenating effects of 'pro-youthful' factors, alternative strategies for rejuvenation should also be considered. Indeed, heterochronic parabiosis and old plasma administration studies have pointed to the existence of 'pro-aging' factors as negative regulators of regenerative capacity in peripheral tissues (Brack et al. 2007, Conboy et al. 2005) and the CNS (Villeda *et al.* 2011, Katsimpardi et al. 2014), as well as cognitive function (Villeda *et al.* 2011). Correspondingly, it has been proposed that mitigating the systemic levels of 'pro-aging' factors in old blood may also provide an effective approach to rejuvenate aging phenotypes (Villeda & Wyss-Coray 2013, Laviano 2014, Villeda et al. 2014). Together, these findings position systemic manipulations altering either 'pro-youthful' or 'pro-aging' factors as exciting new approaches for accessing the plasticity of aging required for tissue and cognitive rejuvenation. Cumulatively, this body of work also provides support for future investigations to test whether the beneficial effects of systemic manipulations extend beyond normal aging to reverse cellular and cognitive decline in those suffering from age-related degenerative disorders such as Alzheimer's disease.

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Figure 1. Interplay between lifespan regulation and brain function. Schematic illustration depicts the unique duality of the brain to be both responsive to systemic lifespan regulation as well as serve as a central regulator of lifespan. Genetic studies have identified the FoxO family of transcription factors, Sirtuins and mTOR signaling pathway as molecular regulators that both promote longevity and mediate critical brain functions known to undergo age-related impairments such as learning and memory. Conversely, the brain has also been demonstrated to promote longevity through neuronal regulation, particularly via the hypothalamus region.

Figure 2. Currently known rejuvenating effects of systemic manipulations on the aged brain. Schematic illustration depicts individual systemic manipulations (exercise, caloric restriction, heterochronic parabiosis and young plasma administration) and their respective effect on each of the three main areas of rejuvenation (neurogenesis, synaptic plasticity and cognitive function). Known enhancements are denoted by a red checkmark, contradictory reports are denoted as a red checkmark with a question mark, and unknown effects are denoted with a question mark.

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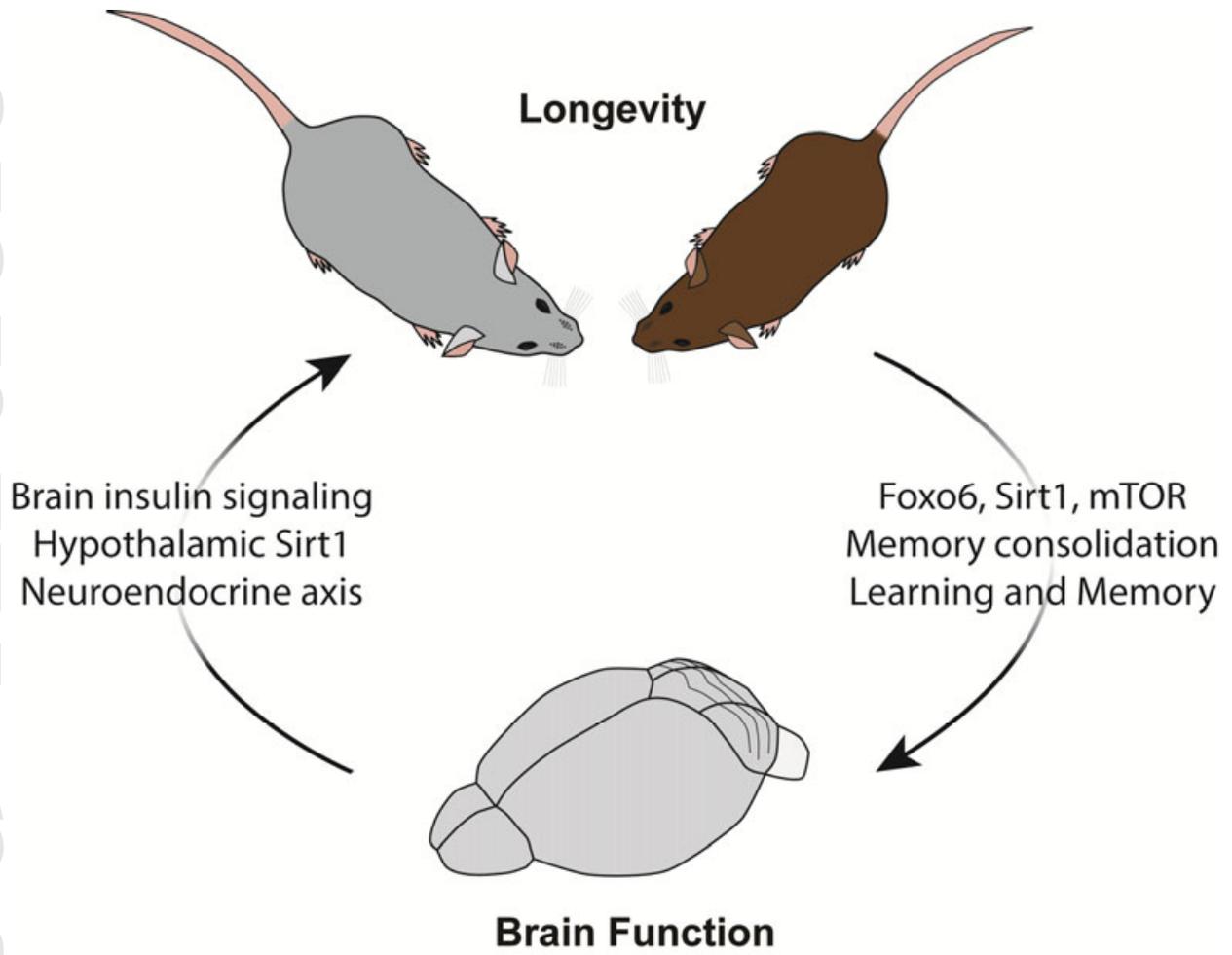
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Table 1: Summary of systemic manipulations and their rejuvenating effects on the aged CNS

	Exercise	Caloric Restriction	Heterochronic Parabiosis	Plasma injection
Neurogenesis & Regeneration	+ neurogenesis in DG van Praag, 2005 Kronenberg, 2006 Wu, 2012 Meriatt, 2012	+ NPSC proliferation in DG + survival of proliferating cells in DG Park, 2013 Lee, 2000 Lee, 2002 Bondolfi, 2004	+ neurogenesis in DG + neurogenesis in SVZ Villeda, 2011 Katsirampali, 2014	
Synaptic Plasticity	+ synaptic plasticity + plasticity-related molecules O'Callaghan, 2009 O'Callaghan, 2009 Meriatt, 2012	+ synaptic plasticity + in plasticity-related molecules Eckles-Smith, 2000 Fontana-Lozano, 2007 Eckles-Smith, 2000 Fontana-Lozano, 2007	+ myelination after spinal cord injury + synaptic plasticity + plasticity-related genes + structural plasticity Ruch, 2012 Villeda, 2014 Villeda, 2014 Villeda, 2014	+ plasticity-related genes Villeda, 2014
Spatial learning & memory	+ spatial learning and memory + object location memory = spatial learning and memory - spatial learning and memory van Praag, 2005 O'Callaghan, 2009 Meriatt, 2012 Singh, 2014 Barnes, 1991 Kumar, 2012	+ spatial learning and memory = spatial learning and memory - spatial learning and memory Piskas & Algeri, 1992 Del-Pan, 2011 Ma, 2014 Beatty, 1987 Bond, 1989 Yanai, 2004		+ spatial learning and memory Villeda, 2014
Cognitive Function	+ attentional processes + executive function + passive avoidance task + cue-discrimination learning + object recognition task + reversal learning = memory and attention processes Hawkins, 1992 Kramer, 1999 Rudek, 2001 Kumar, 2012 Kumar, 2012 Singh, 2014 van Uffelen, 2008a van Uffelen, 2008b	+ non-spatial learning and memory = non-spatial learning and memory Piskas & Algeri, 1992 Fontana-Lozano, 2007 Witte, 2009 Del-Pan, 2011 Taheri, 2014 Merin, 2007	+ odor discrimination Katsirampali, 2014	+ contextual fear conditioning = cued fear conditioning Villeda, 2014 Villeda, 2014

+ increase; = no change; - decrease



	Exercise	Caloric Restriction	Parabiosis	Plasma Injection
 Neurogenesis	✓	✓?	✓	?
 Synaptic Plasticity	✓	✓	✓	✓?
 Cognition	✓?	✓?	?	✓