Osteocalcin in the brain: from embryonic development to age-related decline in cognition

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Abstract

A remarkable, totally unexpected aspect of the bone-derived hormone osteocalcin is that it is necessary for both brain development and brain function in the mouse since its absence results in a profound deficit in spatial learning and memory and an exacerbation of anxiety-like behavior. The regulation of cognitive function by osteocalcin, together with the fact its circulating levels decrease in midlife compared to adolescence in all species tested raised the prospect that osteocalcin may be an anti-geronic hormone that could prevent age-related cognitive decline. As presented in this review, recent data indicate that this is indeed the case and that osteocalcin is necessary for the anti-geronic activity recently ascribed to the plasma of young WT mice. The diversity and amplitude of the functions of osteocalcin in the brain during development and postnatally had long called for the identification of its receptor in the brain, which was also recently achieved. This review presents our current understanding of osteocalcin's biology in the brain, highlighting the bony-vertebrate specificity of the regulation of cognitive function, and pointing toward where therapeutic opportunities may exist.

Introduction

The bone-derived hormone osteocalcin has become synonymous with the skeleton's regulation of energy metabolism, in part because this was the first endocrine function ascribed to this hormone [1]. Although energy metabolism is certainly a critical aspect of osteocalcin biology, whereby it affects [2] in mice, rats and humans [3-12], functions of β-cells [13], myoblasts and likely other cell types, it by no means tells the entire story of osteocalcin. If anything, a hallmark of this hormone is that it regulates a large and continually growing number of physiological functions and developmental processes [14] (Figure 1). This review will focus exclusively on one aspect of osteocalcin biology - its regulation of brain development and functions, which is sometimes overlooked. Yet this function of osteocalcin is of fundamental importance to link this hormone, or its lack thereof, to the biology of aging. The first reason to write a review on this topic at the present time is that the

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biomedical importance of osteocalcin's regulation of several behaviors was recently underscored and linked to the aging of the brain [15]. A second reason is that major progress has recently been made in the elucidation of the molecular basis of osteocalcin's functions in the brain. These advances illustrate the growing reach of this hormone as well as similarities and differences between osteocalcin's peripheral and central functions.

The path to uncover osteocalcin's regulation of brain functions

Demonstrating that anxiety-like behavior and cognition are affected by a molecule made in a peripheral organ has never been an easy or straightforward task. What led to this suspicion in the case of osteocalcin is simply the fact that the extreme passivity of *Osteocalcin-/-* mice [16] could not be explained by any other means. What was already known about osteocalcin when this investigation started was that this molecule, when undercaboxylated, promotes insulin secretion and glucose homeostasis in animals fed a normal diet [1,13,17] and that it favors testosterone synthesis in Leydig cells of the testis but not in follicular cells of the ovary, because its receptor is expressed in the male but not in the female gonad [18-21]. Although low circulating testosterone levels affect several behaviors in male mice [22,23], the poorly defined yet evident passivity was observed in both male and female *Osteocalcin-/-* mice. Hence, it ruled out that this phenotype was due to a defect in sex steroid hormone synthesis, since this process is not affected by osteocalcin in female mice. Likewise, it became clear very quickly that the passivity of *Osteocalcin-/-* mice was not secondary to their abnormal glucose metabolism either, since this passivity was not seen in mice lacking *Gprc6a*, the receptor used by osteocalcin to favor insulin synthesis in pancreatic β-cells [13,24-26]. The fact that *Gprc6a-/-* mice had none of the behavioral abnormalities we will describe has been important throughout this investigation for several reasons. A first one is that it allowed us to uncover a decrease in muscle function during exercise caused by the absence of osteocalcin signaling through Gprc6a in myoblasts [2] and to distinguish this from the passivity caused by the absence of osteocalcin signaling through a different receptor present in the brain. This lack of an obvious explanation for their abnormal passivity led to the suspicion that these phenotypes of the *Osteocalcin-/-* mice might indicate that osteocalcin normally signals and functions in the brain. What added considerable credence to this notion is that osteocalcin had been shown in the original study of its central function to cross the blood brain barrier since peripheral delivery of uncarboxylated osteocalcin in *Osteocalcin-/-* mice resulted in an accumulation of osteocalcin in specific regions of the brain, mainly in the midbrain and brainstem [24]. In contrast, when delivered peripherally, carboxylated osteocalcin crossed the blood brain barrier far less efficiently [24]. This observation led us to a remarkable string of findings.

Osteocalcin's regulation of anxiety and cognition in adult mice

Moving from the entire animal to the molecular level, we observed that adult mice lacking *Osteocalcin* displayed a substantial increase in anxiety-like behavior compared to wild-type (WT) littermates from a young age (3-months-old), and had a major deficit in learning and memory. Written as such, this does not do justice to the severity of the phenotype observed in *Osteocalcin-/-* mice of both sexes. Indeed, in the Morris water maze test [27], *Osteocalcin-/-* mice were simply unable to learn the location of the platform and find it in a timely fashion.

This finding identifies osteocalcin as a peripheral hormone exerting one of the strongest influences on spatial learning and memory.

Anatomically, the brains of the *Osteocalcin-/-* mice are consistently smaller than those of their WT littermates [24]. The most overt abnormality however, is seen in the hippocampal region where the area covered by the dentate gyrus is significantly 30% smaller in *Osteocalcin-/-* than in WT mice, and the corpus callosum, which serves as a bridge between the hippocampi of each hemisphere [28], is often missing in the *Osteocalcin-/-* mice [24]. Biochemically, the content of all monoamine neurotransmitters [29], i.e, serotonin, dopamine and norepinephrine are significantly lower by 20-50% in the midbrain and brainstem of *Osteocalcin-/-* compared to WT mice. At the same time, the accumulation of GABA, an inhibiting neurotransmitter [30], in the same areas of the brain was increased by 15-30% in *Osteocalcin-/-* mice. These abnormalities are secondary, at least in part, to transcriptional events since the expression of the genes needed for the synthesis of monoamine neurotransmitters [31-33] is decreased by 15-50%, whereas the expression of two genes necessary for GABA synthesis, *Gad1* and *Gad2* [34], is increased by almost 2-fold in the brainstem of *Osteocalcin-/-* mice. Even though the receptor for osteocalcin in these neuronal cells was not identified at the time these experiments were performed in 2013, we observed that osteocalcin binds to neurons of the CA3 region of the hippocampus, to neurons of the ventral tegmental area, a nucleus located in the midbrain which is enriched in dopaminergic neurons [35], and to the dorsal and medial raphe that contain serotonergic neurons [36] (Figure 2). Accordingly, in electrophysiological assays, osteocalcin could activate the action potential frequency of neurons by almost 2-fold in the brainstem and locus coeruleus [24].

Two experiments led to the conclusion that all these abnormalities reflect the absence of osteocalcin signaling in the brains of adult mice. The first one is that the delivery of osteocalcin in the brain through intracerebroventricular infusion, at a dose that does not result in any measurable leakage of osteocalcin in the general circulation, fully corrects anxiety-like behavior and memory phenotypes, as well as gene expression abnormalities. Second, an osteoblast-specific deletion of *Osteocalcin* performed in adult mice results in the same increased anxiety-like behavior phenotype and in a memory defect, albeit these are less severe than what is observed in *Osteocalcin-/-* mice. Neurotransmitter contents and gene expression were also significantly affected in mice harboring a post-natal and osteoblast-specific deletion of *Osteocalcin*. As expected, the only phenotype that could not be recapitulated in mice lacking Osteocalcin only postnatally was developmental, i.e. their hypomorphic hippocampi [15].

Maternal osteocalcin contributes to brain development and the acquisition of cognitive function in the offspring

The normal brain development observed in mice induced to lack *Osteocalcin* only in osteoblasts and when they reach adulthood, along with their more modest deficit in spatial learning and memory, were two reasons to suspect that osteocalcin could influence the brain during embryonic development. Since one cannot detect any osteocalcin expression in the

mouse embryo before E17.5 [37,38], this hypothesis implied that maternal osteocalcin might cross the placenta and the blood brain barrier to execute this putative function.

This hypothesis turned out to be correct. Maternal osteocalcin begins to cross the placenta between E14.5 and E15.5 [24], at a time when development of structures such as the hippocampus is in full speed [39] (Figure 3). A complicated but efficient breeding strategy could establish unambiguously that virtually all the osteocalcin that one could find in an embryo before E18.5 is of maternal origin. Moreover, we could also show that maternally derived osteocalcin is needed to favor fetal neurogenesis and prevent neuronal apoptosis [24]. Maternal osteocalcin is also necessary for the establishment of spatial learning and memory in adult mice. Indeed, delivering uncarboxylated osteocalcin or vehicle to *Osteocalcin-/-* mothers crossed with *Osteocalcin-/-* fathers throughout pregnancy, and testing the behavioral performance of their progeny at three months of age showed that maternal osteocalcin contributes to a substantial extent but does not completely explain the deficit in spatial learning and memory that is observed in adult *Osteocalcin-/-* mice [24].

What did we learn, what did we not learn?

Taken together, this series of observations revealed several important and novel notions about brain physiology and brain development. Moreover, given where osteocalcin is synthesized, taken at face value, these observations also enriched bone physiology. If one looks at brain physiology, these findings showed unambiguously that peripheral hormones, and certainly one made in bone, osteocalcin, exert a rather powerful influence on neuronal activity, gene expression, neurotransmitter synthesis and ultimately behaviors such as anxiety-like behavior, spatial learning and memory. This notion is of fundamental importance, as too often we conceive and study functions of a given organ omitting the fact that this organ receives many, most of them still unknown, inputs from other organs. Underscoring the potential medical influence of this work, two retrospective studies performed in human subjects [40,41] after the initial paper of Oury et al., was published have suggested a correlation between decreased circulating osteocalcin levels and poor cognitive performance in healthy aged individuals. We are fully aware that these studies, because of their retrospective nature, can only be seen as encouraging, and that projective studies are now needed. In terms of brain development and physiology, this work showed that one molecular underpinning of the so-called Barker effect [42,43] (the maternal influence on neuropsychological functions of the offspring) may be a relative lack of maternal osteocalcin. This is important if we consider that the Barker effect also includes the maternal influence on energy metabolism in the offspring, as energy metabolism is one of the main physiological processes regulated by osteocalcin [44-49].

How does one place these data in a broader context of bony vertebrate-specific biology? Two aspects of osteocalcin biology are particularly helpful in that respect. The first one is that when to pre-pubertal mice and human beings, circulating levels of osteocalcin decrease steeply before 9 months of age in the mouse, and before age 30 and age 45 in women and men respectively, without reverting to levels observed during puberty [2]. In other words, circulating osteocalcin levels are high during life when cognitive functions are at their peak as well. Importantly for our purpose, this is a specific feature of osteocalcin since other bone

specific hormones like lipocalin 2 (data not shown) or sclerostin [50] circulating levels increase with age. A second feature of osteocalcin that is worth mentioning is that osteocalcin circulating levels surge during exercise, a biological event that is also known to improve cognitive function [2,51-55]. These findings raise the following interconnected questions: (A) could the decrease in cognitive function that occurs during aging be due at least in part to the plummeting of circulating osteocalcin levels? And (B) could osteocalcin be sufficient to correct age-related cognitive defects?

Besides these biomedical questions, there is also a molecular question that needs to be answered - how does osteocalcin signal in the brain? Indeed, the fact that *Gprc6a* is not expressed anywhere osteocalcin signals in the brain, and does not mediate osteocalcin signal in the brain, has been initially a huge strength of this project as it allowed ruling out the possibility that central functions of osteocalcin were secondary, even in part, to peripheral ones. Once this point was made, the task remained to understand how osteocalcin signals in the brain, i.e., to identify its receptor in the brain. This could serve several purposes of varying nature. One purpose of course is to achieve a better understanding of how extracellular cues coming from the periphery can affect brain development and functions. A second one is, depending of the pattern of expression of this receptor, to possibly broaden the portfolio of central functions of osteocalcin. A third purpose, as important as the other two, dependent on the nature of this receptor and of its pattern of expression, could be to harness its biology for therapeutic purposes.

Osteocalcin is necessary and sufficient to correct age-related decline of cognitive function

The most fundamental question raised by this first study in the mouse, establishing bone as a regulator of brain development and functions via osteocalcin is to determine how useful these findings could be or could become from a medical point of view, especially in the aging population. This question stems from the severe behavioral phenotype of the *Osteocalcin-/-* mice and other types of observations. For instance, with the progressive aging of the general population [56-58] in developed countries but eventually all over the world, there is an urgent need to find ways to prevent age-related cognitive decline and other age-related diseases affecting brain functions [59-63]. Moreover, it has been shown that for every function regulated by osteocalcin, delivery of exogenous uncarboxylated osteocalcin can improve the age-related decline of these functions [2,24]. One reason for this could be that circulating levels of osteocalcin plummet beyond midlife in all species tested [2,64,65]. Finally, the aforementioned clinical studies [40,41], which suggested an association between circulating osteocalcin levels and cognitive performance, were another reason to ask whether osteocalcin could be sufficient to restore cognitive functions in older mice. We have begun to address this question in animal models in several ways.

The first one was to use the assay popularized by Villeda et al [66] published in 2014, showing that repeated injections of plasma obtained from 3-month-old mice could improve cognitive function in older mice, i.e in 16-month-old mice. We need to add a precision here, we as well as many other laboratories are able to make mice live beyond 24 months of age in the

confined environment of a modern animal facility, and this may be used as a definition of aging. However, in the wild, the definition of aging for a mouse is different as it is dubious that mice beyond 9- or 12-months would have the necessary fitness to survive in their somewhat hostile environment. In any case, after reproducing the results of Villeda et al., we showed that in contrast, injections of plasma from 3-month-old *Osteocalcin-/-* mice into 16-month-old WT mice could not improve cognitive function or anxiety-like behavior as plasma from 3-month-old WT mice did. There are many possible factors that could explain these results, yet they established on genetic grounds that osteocalcin is necessary for the beneficial effect of plasma from young mice on several brain functions in older mice. To rule out that this lack of efficacy of plasma from young *Osteocalcin-/-* mice in improving the behavioral performance of older mice represented a developmental effect, and to link osteocalcin itself to the beneficial effect of plasma from young WT mice, older mice were injected with plasma from young *Osteocalcin-/-* mice, which recombinant uncarboxylated osteocalcin had been added ("spiked plasma") (Figure 4a). That this spiked plasma could improve cognitive function and decrease anxiety-like behavior in older WT mice to the same extend as plasma from young WT mice, was a strong indication that osteocalcin was not only necessary but also sufficient to improve cognitive function and to hamper anxiety-like behavior in adult mice. In a mirror image experiment, depleting every form of osteocalcin from the plasma of young WT mice though immunological means, removed from WT plasma its ability to improve cognitive functions and to decrease anxiety-like behavior [15] (Figure 4b).

The most direct evidence that osteocalcin is sufficient to rescue age-related cognitive decline and to decrease anxiety-like behavior, independently of any developmental effect, came from the following experiment. Since osteocalcin crosses the blood brain barrier [24] we implanted subcutaneously mini osmotic pumps delivering uncarboxylated osteocalcin or vehicle for 60 days in 10- or 14-month-old mice. Vehicle- and osteocalcin-treated were analyzed when they were 12-or 16-month-old. What was observed is that chronic delivery of uncarboxylated osteocalcin in 10-or 14-month-old mice fully rescued their deficit in cognitive function and decreased their anxiety-like behavior [15].

In exploring the molecular bases of this remarkable ability of osteocalcin to rescue age related cognitive decline, several lines of experimental evidence pointed toward brain-derived neurotrophic factor (BDNF) a molecule well known to favor hippocampal dependent memory [55,67-70], as a critical mediator of osteocalcin's regulation of cognitive function. The first was observed when monitoring multiple parameters of the transport of BDNF-containing dense-core vesicles in embryonic rat neurons in microchambers [71]. In this setting, uncarboxylated osteocalcin was able to stimulate the dynamic of BDNF vesicle transport to synapse in rat neurons. This translated in an increase in velocity and in the number of BDNF vesicles moving to the synapses. In cell culture, osteocalcin treatment of primary hippocampal neurons with osteocalcin stimulated *Bdnf* expression. A second piece of evidence supported in vivo that BDNF is a mediator of osteocalcin in the brain. Stereotaxic injection of uncarboxylated osteocalcin in the anterior hippocampi could increase BDNF accumulation in the hippocampus. Lastly, peripheral injections of uncarboxylated osteocalcin increase *Bdnf* expression in hippocampi of 16-month-old mice. Similar to this, injection of plasma from 3-month-old mice to 16-month-old mice increased BDNF

accumulation in the hippocampi of 16-month-old mice. This beneficial effect of young plasma on BDNF accumulation was abolished when plasma from 3-months-old *Osteocalcin-/-* mice was injected to 16-months-old WT mice [15].

Identification of a receptor mediating osteocalcin's regulation of cognitive function and anxiety-like behavior

The fact that osteocalcin is not only necessary but sufficient to improve cognitive decline at any age but even more in aged mice, and to reduce anxiety-like behavior, made it imperative to identify and characterize a receptor mediating the functions of this hormone in the brain. To tackle this problem, and in trying to avoid a long and often dangerous fishing expedition, three criteria were used to identify what could be a candidate receptor of osteocalcin in the brain. First, based on what has been observed in many fields, we posited that a new receptor for osteocalcin should be a G-coupled receptor (GPCR) that should belong to the same class as Gprc6a, the class C family [72,73]. This criterion, because the class C orphan GPCR list is rather small [74], considerably reduced the size of the pools one had to explore. A second criterion was that this orphan GPCR should be expressed in an area where it had been previously shown that osteocalcin binds. The third criterion was that this orphan GPCR should not be expressed in any of the cell types where Gprc6a mediates osteocalcin's signaling [2,13,17,18]. When these three criteria were used as a filter, only one orphan class C GPCR fulfilled all of them: Gpr158. It remained then to demonstrate that Gpr158 was the actual receptor of osteocalcin.

Gpr158's pattern of expression was certainly consistent with this notion since it is expressed in the CA3 region of the hippocampus where osteocalcin binds; in this area of the brain, *Gpr158* is expressed in pyramidal neurons and not in astrocytes. The fact that Gpr158 was more abundant in *Osteocalcin-/-* than WT hippocampi was a further suggestion that it could be a receptor of osteocalcin in the hippocampus. Like Gprc6a, Gpr158 is expressed in humans in the same structure and regions where it is expressed in the mouse [75,76] (Figure 5). All the other evidences that were gathered in support of this hypothesis were of genetic nature and were obtained either ex vivo or in vivo [15].

For instance, local delivery of osteocalcin in the anterior hippocampus induced c-Fos accumulation, a marker of neuronal activity [77,78], in neurons of the CA3 region in WT mice but not in *Gpr158-/-* mice. Osteocalcin could bind a complex containing Gpr158 and the Gαq protein in solubilized membranes obtained from *Osteocalcin-/-* hippocampi, whereas the quantity of pulled down by osteocalcin was considerably reduced when using solubilized membranes obtained from *Gpr158-/-* hippocampi. In agreement with this observation, osteocalcin increased the accumulation of IP3, a second messenger used by GPCR [79,80], in WT but not *Gpr158-/-* hippocampal neuron whereas it consistently failed to increase cAMP accumulation in hippocampal neuron. In electrophysiological assays, in whole-cell current clamp recording osteocalcin enhanced action potential frequency in pyramidal neurons of the CA3 region of the hippocampus of WT but not of *Gpr158-/-* hippocampi, this effect of osteocalcin was nearly abolished when using an IP3 receptor antagonist (Figure 6). An

analysis of long term potentiation [81,82] (LTP) verified that osteocalcin signals in WT but not in *Gpr158-/-* hippocampal slices [15].

In vivo, many arguments of genetic nature also indicated that Gpr158 is a receptor for osteocalcin in the brain. For instance, whether we analyzed mice lacking *Gpr158* only in the forebrain or in all cells we observed the same severe deficit in hippocampal-dependent memory [83] in mice lacking this receptor [15]. Likewise, *Gpr158-/-* mice manifested more severe anxiety-like behavior than WT littermates. That neither cognitive deficit nor anxiety-like behavior could be corrected by chronic delivery of osteocalcin in *Gpr158-/-* mice established that Gpr158 is necessary to mediate osteocalcin's regulation of cognitive function and anxiety-like behavior. Two additional lines of evidence, of genetic nature, demonstrated that it is indeed the case. The first was to show that stereotaxic injection of osteocalcin in the anterior hippocampi could improve hippocampal dependent memory in WT mice but not in mice in which Gpr158 accumulation has been decreased by a small hairpin RNA interference strategy [84]. Second, compound heterozygous mice lacking one allele of *Gpr158* and one allele of *Osteocalcin* (*Gpr158+/-; Osteocalcin+/-*) demonstrated the same cognitive deficit as that observed in *Gpr158-/-* or in *Osteocalcin-/-* mice whereas single heterozygous mice behaved like WT mice [15].

Independently of this body of work that identified Gpr158 as a receptor mediating osteocalcin regulation of cognitive function and anxiety-like behavior, several other important observations were made in the course of this investigation. One is that, as we had long surmised, osteocalcin function in the brain may not be limited to the hippocampus and the ventral tegmental area of the midbrain. Indeed, a systemic analysis of *Gpr158* expression showed that it is also expressed in the somatosensory area, motor and auditory areas of the cortex, the piriform cortex and the retrosplenial area, where it was not known that osteocalcin binds. A second more surprising observation that needs to be interpreted cautiously, is that *Gpr158* is apparently not expressed in serotonergic neurons of the dorsal and median raphe of the brainstem where osteocalcin binds [15]. This suggests the possibility that a third receptor for osteocalcin may exist.

Conclusions

What was illustrated through this work is a classic and fundamental principle of physiology; no organ, as noble as it may be, is an island that develops and functions at the exclusion of other organs. In our current era of biology, we have developed the tools to explore molecular events so effectively, and yet mouse genetics has given us the power to uncover broader physiological phenomena that have remained hidden for so long. What we did not know and could not have anticipated a priori is that the skeleton, of all organs, would secrete a hormone that exerts such a powerful influence on the brain in the mouse and it seems from retrospective studies probably in humans. Here, one should focus first and foremost on the salient features of osteocalcin and how it could help medicine. it is impossible to address these questions without considering the natural history of osteocalcin, whose circulating levels decrease around mid-life in all species tested. This is a fundamental feature of osteocalcin in view of the fact that this hormone is necessary to maintain at an optimum level cognitive function and to decrease anxiety-like behavior. Maybe more importantly

from a medical point of view is the notion that osteocalcin is necessary and sufficient to correct age-related cognitive defects and to hamper anxiety-like behavior. Together these features identify osteocalcin as an anti-geronic hormone that joins TIMP2 [85] on the list of anti-geronic molecules. Having identified a receptor for this hormone in the brain now raises the prospect that this interaction between osteocalcin and Gpr158 could be used to treat various age-related degenerative conditions affecting the brain. This may even be more important going forward since the pattern of expression of *Gpr158* suggests that we are not yet aware of all the functions of osteocalcin in the brain.

The physiology and aging of the brain is only one side of the coin. The other aspect one has to recognize is that osteocalcin is one of the first, if not the first, molecular substratum for the long-suspected maternal influence on brain development. How important the role of osteocalcin is in brain development is not yet fully known, in part because we do not know all the functions of osteocalcin in the brain, and in part because we also do not know the pattern of expression of Gpr158 in the developing brain. These notions, and the possibility that a third receptor for osteocalcin may exist, represent the most concrete challenges in front of us.

Acknowledgments

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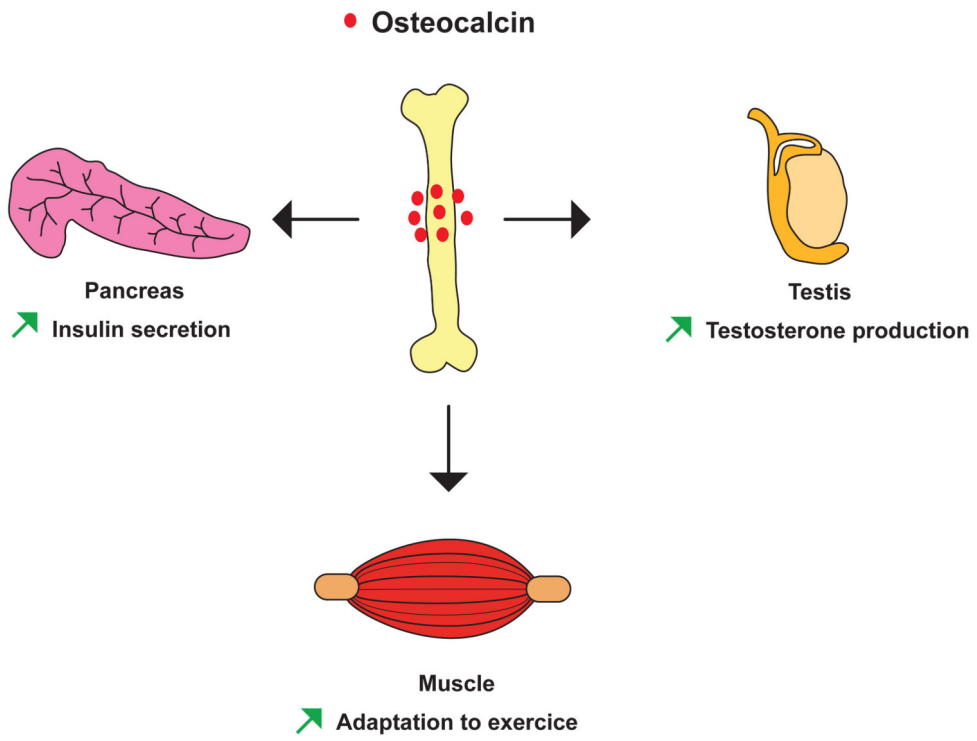
Figure 1. Osteocalcin functions in peripheral organs
Schematic representation of the physiological functions regulated by osteocalcin.
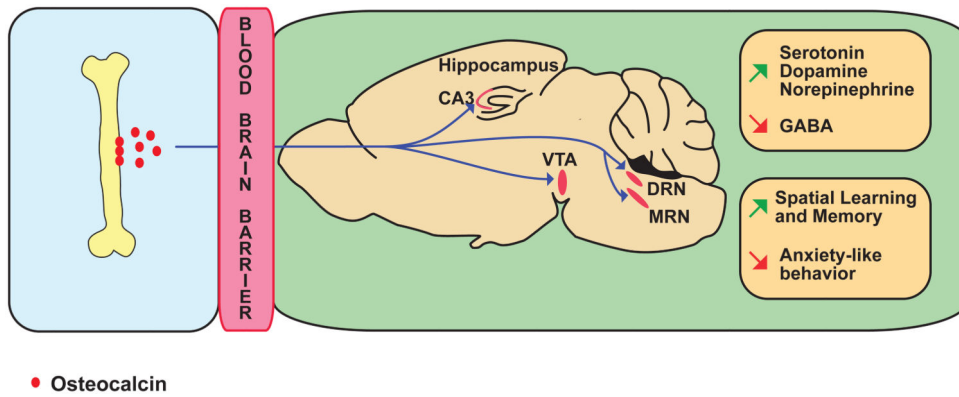
• Osteocalcin

Figure 2. Osteocalcin promotes spatial learning and memory and prevent anxiety-like behavior
Osteocalcin is made in bone, uses the general circulation to cross the blood brain barrier (BBB) and then binds neurons from the CA3 region of the hippocampus, the ventral tegmental area (VTA) of the midbrain, and dorsal raphe (DRN) and medial raphe (MRN) nuclei of the brainstem. Osteocalcin acts on these neurons through transcriptional events to increase the synthesis of all monoamines neurotransmitters and decrease that of GABA. Osteocalcin promotes spatial learning and memory and prevents anxiety-like behavior.
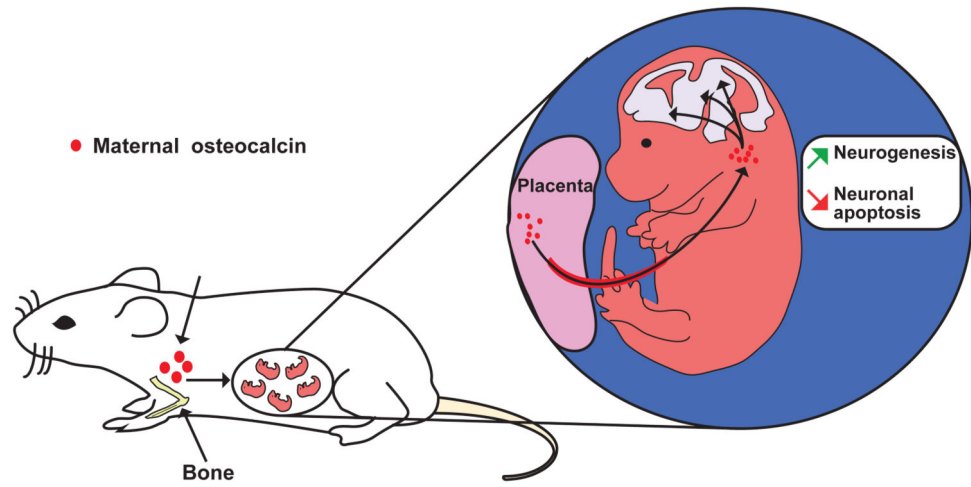
Figure 3. Maternal osteocalcin acts directly in the offspring to regulate embryonic brain development

Maternal osteocalcin crosses the placenta, promotes neurogenesis and prevents neuronal apoptosis in the hippocampal region of the offspring.
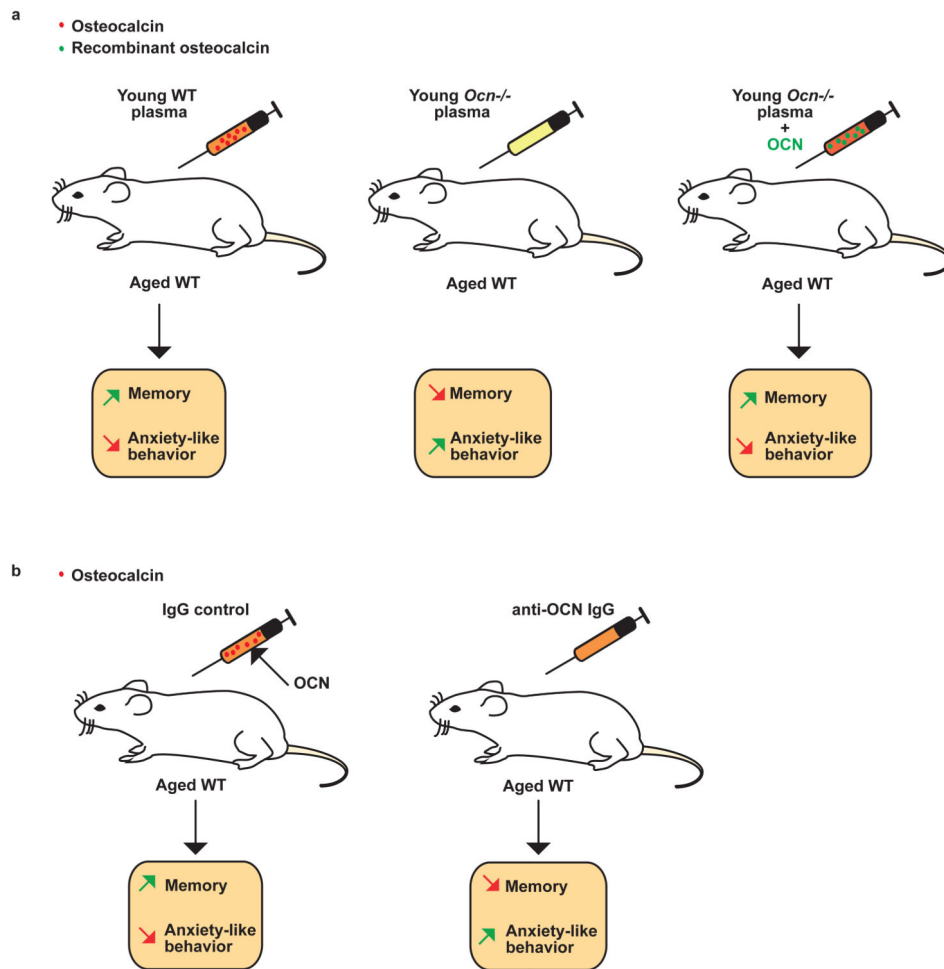
Figure 4. Osteocalcin is sufficient to improve cognition in aged mice
(**a**) Injections of plasma from young wild-type (WT) mice to aged WT mice improve memory performance and prevent anxiety-like behavior. This does not occur when plasma from young *Osteocalcin-/-* (*Ocn-/-*) mice is used instead of plasma from young WT mice. When *Ocn-/-* plasma is spiked with mouse recombinant osteocalcin, the ability of this plasma to improve cognitive functions is restored to same extend as when using plasma from young WT mice. (**b**) Plasma from young WT mice immunodepleted of osteocalcin cannot enhance cognition in aged mice.
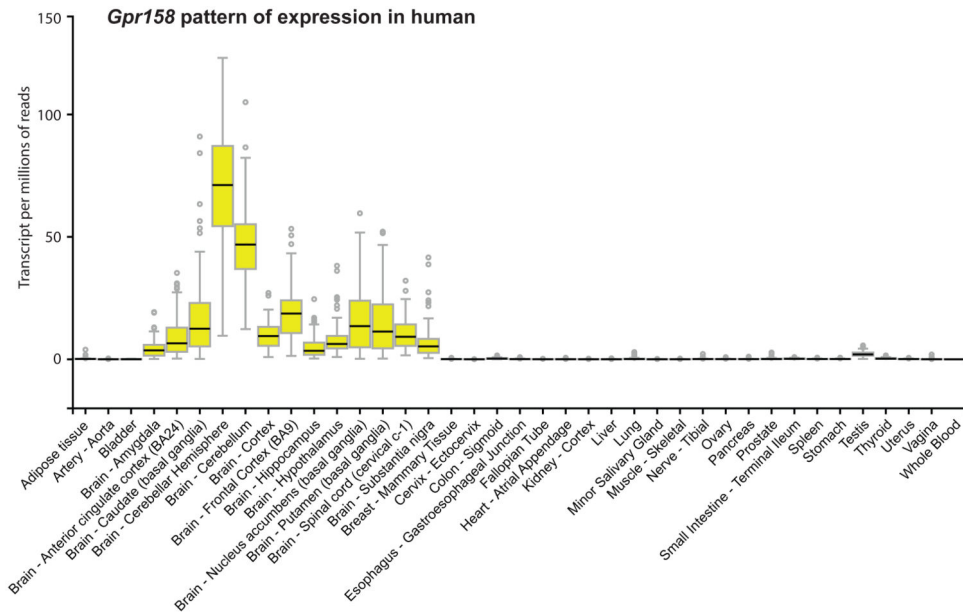
Figure 5. Gpr158 pattern of expression in human tissues
Gpr158's pattern of expression obtained from the GTEX database (www.gtexportal.org).
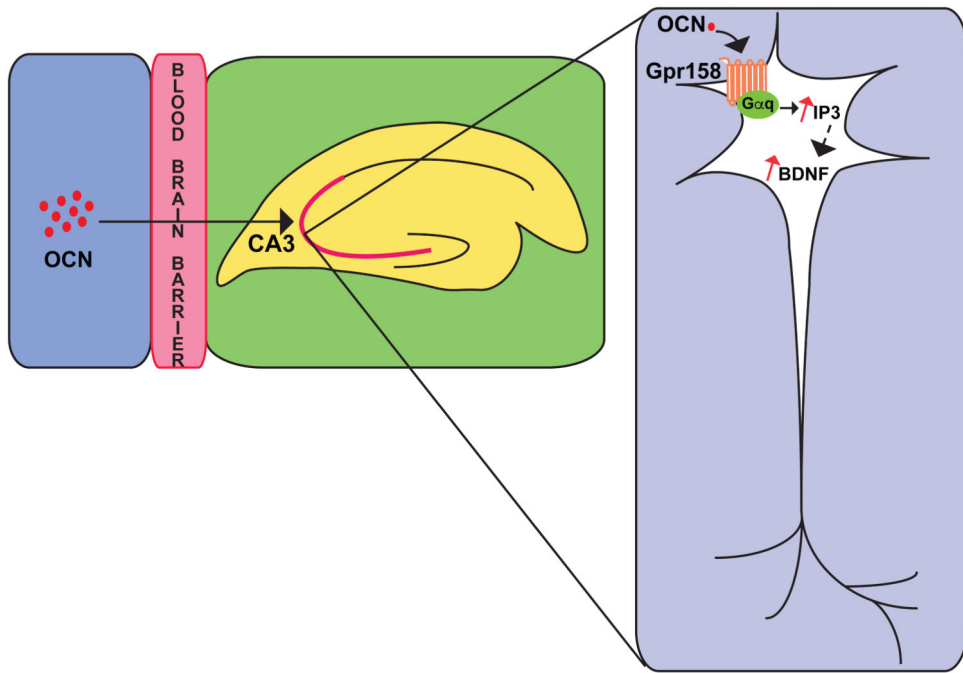
Figure 6. Gpr158 mediates osteocalcin's functions in the brain
Osteocalcin binds Gpr158 in pyramidal neurons of the CA3 region of the hippocampus. This interaction promotes an increase in the accumulation of IP3 and BDNF in neurons.