

Bone, brain & beyond

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Abstract In the past 15 years, the field of physiology has been radically challenged by landmark studies using novel tools of genetic engineering. Particular to our interest, the reciprocal interactions between the skeleton and the nervous system were shown to be major ones. The demonstration that brain, via multiple pathways, is a powerful regulator of bone growth, has shed light on an important central regulation of skeletal homeostasis. More recently, it was shown that bone might return the favor to the brain through the secretion of a bone-derived hormone, osteocalcin. The skeleton influences development and cognitive functions of the central nervous system at different stages throughout life suggesting an intimate dialogue between bone and brain.

Keywords Bone · Brain · Osteocalcin · Whole-organism homeostasis · Behavior · Integrative physiology

1 Introduction

Throughout life, the endocrine system is instrumental in the maintenance of whole organism physiology and, besides the

central nervous system, represents a major communication system of the body. Via the secretion of hormones, it influences various physiological functions such as reproduction, energy metabolism, bone integrity, growth and development as well as cognitive functions. The importance of the hormonal signals on whole-organism physiology is based on one of the major principles of integrative physiology, the mutual dependence between organs. In other words, all organs need to communicate with each other to maintain physiological functions at optimal levels. This paramount notion was coined by the concept of the milieu interieur defined by Claude Bernard and forged by Walter Bradford Cannon describing the concept of whole-body homeostasis [1, 2]. The emergence of genetically engineered mouse models has profoundly rejuvenated the genuine and fundamental notion of whole-body physiology and allowed us to identify unexpected crosstalk between organs.

The endocrine system includes various organs such as the pancreas, kidneys, adrenal glands, gonads, thyroid, parathyroids, and even fat which are all sources of hormones. In the past decade, bone has emerged as one of them. Beyond the classical view of the skeleton describing bone as a relatively static tissue, multiple genetic-based studies have shed light on the growing physiological importance of this organ in whole-body homeostasis [3–6]. In fact, the skeleton is not only a receptacle for external inputs but it secretes at least two hormones necessary for whole-body homeostasis. First, through the secretion of fibroblast growth factor 23 (FGF23), bone regulates phosphate and calcium metabolism [3, 7, 8]. Second, the skeleton secretes an osteoblast-derived molecule, osteocalcin (Ocn), that does not act directly on bone but that rather, via a feedback loop control mechanism, affects a variety of physiological functions in both mice and humans. Indeed, Ocn favors proliferation of pancreatic β -cells, secretion of insulin by these cells and insulin sensitivity in both muscle and white adipose tissue. As a result, Ocn promotes glucose

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homeostasis, enhances energy expenditure and prevents the appearance of glucose intolerance induced by high fat diet [4, 5, 9, 10]. Moreover, Ocn increases the synthesis of testosterone by Leydig cells of the testis and thereby enhances male fertility [6, 11–13]. Recently, it has been shown that Gprc6a, a G-protein coupled receptor that belongs to the calcium-sensing family, mediates endocrine functions of Ocn on glucose homeostasis and testosterone production [6, 11–14]. In addition, a loss-of-function mutation in this receptor causes male sterility and glucose intolerance in humans, suggesting the conservation of the endocrine function of Ocn signaling [14, 15]. The demonstration that bone affects such a variety of physiological functions reinforced the concept of mutual dependence between organs and it suggests that the skeleton is an endocrine organ integrated in a larger network essential for the regulation of whole-body homeostasis.

A growing body of evidence indicates that hormonal signals converge on the central nervous system and that the brain integrates hormonal signals from the periphery. In fact, the brain expresses receptors for many hormones including insulin, insulin-like growth factor, ghrelin, thyroid hormone, the parathyroid hormone and leptin, which, taken up from the blood, help shaping the developing brain, affect behavioral functions, neuronal activity, neurogenesis, synaptic activity and contribute to age-related cognitive decline [16]. In response to environmental changes such as stress and changes in our biological clocks during the nycthemeron, hormones enter the blood and travel to the brain and other organs to fulfill their function as far-reaching messengers. In the brain, hormones alter the production of gene products that participate in synaptic neurotransmission as well as the structure of brain cells. As a result, the circuitry of the brain and its capacity for neurotransmission are changed. In this way, the brain adjusts its performance and the control of behavior in response to environmental signals. This fundamental importance of hormonal signals in brain function is well illustrated by the contribution of sex steroid hormones to the modulation of neuronal differentiation, brain structure and cognitive functions both during the prenatal period and periods that are characterized by massive sex steroid changes [17–19], as well as by more recent studies demonstrating that blood derived from young mice can rejuvenate adult hippocampal neurogenesis and age-related cognitive decline [20]. Together, these data suggest a more global role of each organ in the function of the body. In other words, each organ does not only perform its specific functions but also regulates far-reaching processes of the organism by secreting organ-specific factors into the bloodstream.

The growing importance of bone as an endocrine organ and recent advances in physiology have enabled to bridge bone and brain physiologies. Since the early developments of anatomy and histology, the skeleton is known as a highly innervated tissue [21], an evidence

that suggests an intimate dialogue between neural and skeletal tissues. However, the vision of two separated organs has only been challenged in the early 2000s [22]. In the past 15 years, a combination of mouse and human genetic studies have identified the brain as a powerful regulator of bone growth [22–24]. Following an Ariadne's thread, it was first shown that leptin, a powerful inhibitor of bone remodeling, is acting through a central relay to increase sympathetic tone and down-regulate bone remodeling [22, 24]. However, numerous studies have shown that the influence of the brain on the skeleton is even more complex and tightly linked, involving various neural mediators that will be discussed in this review.

Given that bone affects multiple physiological functions by virtue of a feedback loop control, together with the powerful inhibition of bone mass accrual exerted by the brain [22–24], it was recently hypothesized that bone might signal back to the brain in order to modulate the extent of the central inhibition of bone mass accrual. Testing this possibility, a functionally relevant dialogue between the bone and the brain relying on Ocn was recently uncovered, which occurs at different stages throughout life. Postnatally, Ocn crosses the blood–brain barrier, enhances the synthesis of several neurotransmitters, and inhibits synthesis of γ -aminobutyric acid (GABA). In addition, Ocn influences adult hippocampal neurogenesis (AHN), a process of generating newborn neurons throughout lifetime that is associated with age-related cognitive decline, and, hence, may explain the decline of cognitive functions during aging [25]. Hence, most likely, the absence of Ocn in adult animals hampers several cognitive functions. In fact, Ocn is important for the regulation of anxiety and depression and for optimal spatial learning and memory. During embryonic development, maternal Ocn crosses the placenta and regulates brain development and functions. In particular, absence of the maternal pool of osteocalcin induces enlargement of the lateral ventricles of the brain and increases the number of apoptotic cells in the hippocampus. These abnormalities lead to an impairment of spatial learning and memory of the offspring. These structural and functional brain deficits during development and adulthood, induced by the absence of Ocn, established Ocn as an endocrine messenger that influences the central nervous system and suggests an important crosstalk between bone and brain.

In this review, we will summarize the advances that have been made in the last 15 years in the field of neuroskeletal physiology, from the landmark discovery of the central regulation of bone mass [22] until the recent discovery that the skeleton is a determinant of brain development, neuronal structure and behavioral functions [25]. Finally, we will infer

the observations from animal models to human studies in pathologic conditions.

2 The central regulation of bone mass

Bone is an organ essential for locomotion and is defined primarily by its mechanical and scaffolding properties. Based on these characteristics, it is crucial for vertebrates to maintain a constant bone mass and high quality of bone throughout life. This is achieved by a biphasic process called bone remodeling, a continuous process of skeletal renewal. This continuous bone turnover requires a significant amount of energy for the recruitment of osteoblasts and the deposition of extracellular matrix. The skeleton has been described for long as a highly innervated tissue [21]. Sensory and autonomic neurons pass through Havers' and Volkmann's canals and innervate bone. Nevertheless, the importance of central regulation of bone physiology has only recently started to be deciphered. Brain regulates bone mass either by the direct action of the neurotransmitters that it produces or by acting as a mediator in processing peripheral hormones signal, such as leptin that originates from adipose tissue. In this section, we will present an overview of the central regulation of bone physiology.

2.1 Identification of the central regulation of bone mass: a detour from leptin to bone

The first insight into the central regulation of bone mass was assessed following the assumption that bone remodeling is a highly energy demanding process and might be coordinated with other physiological processes as diverse as energy metabolism, sleep–wake cycle and reproduction. Clinical evidence supported the notion of such a physiological crosstalk, e.g. a near-total arrest of growth in childhood and low adult bone mass in patients with anorexia nervosa [26, 27]. To explore the assumption of such a crosstalk between bone and highly energy demanding processes, work was initiated by Ducy et al., [22] investigating leptin, an adipocyte-specific hormone identified for its ability to regulate food intake and to favor energy expenditure and reproduction [28–30]. This study led to the seminal observation that mice lacking leptin (*ob/ob* mice) or its receptor (*db/db* mice) displayed a high bone mass due to a massive increase in bone formation [22, 23, 31]. Although *in vitro* studies have shown that leptin, under certain conditions, can affect osteoblast functions directly [32], genetic evidence indicates that leptin inhibits bone mass accrual mainly by acting on the brain, as it is the case for other functions of this hormone [33]. Indeed, intracerebroventricular (ICV) injection of leptin decreased bone architecture by hampering osteoblast activity. Conversely, when leptin was ICV injected in *ob/ob* mice, it normalized their bone mass to WT levels [22, 24]. Furthermore, neuron-specific deletion of

the leptin receptor (ObRb) recapitulates the bone phenotype of *ob/ob* mice whereas an osteoblast-specific deletion does not [34].

Analyzing the site of action of leptin in the brain, it was shown that the antiosteogenic action of leptin acts through the ventromedial hypothalamus (VMH) [24]. Indeed, gold thioglucose (GTG)-mediated chemical ablation of VMH neurons did not affect body weight but resulted in a high bone mass phenotype that could not be corrected by ICV infusion of leptin [24]. Furthermore, both *ob/ob* mice lacking leptin as well as GTG-treated mice had low levels of catecholamines, indicating an interaction with the sympathetic nervous system. Indeed, generation of knock-out mice for the beta-2 adrenergic receptor (*Adrβ2R*^{-/-} mice), one of the receptors mediating catecholamine norepinephrine action in the cells, produced a phenocopy of bone features seen in *ob/ob* mice, suggesting that the sympathetic tone regulates bone remodeling. In contrast, bone mass remained unchanged in *Adrβ2R*^{-/-} mice when leptin was infused ICV, suggesting that the sympathetic nervous system, through *Adrβ2R*, is necessary and sufficient to mediate central leptin action on bone mass accrual. At the cellular level, the triggered biochemical mechanisms involved an imbalance between the phosphorylation levels of cAMP response element-binding protein (CREB) and of the activating transcription factor 4 (ATF4). After activation by its ligand norepinephrine, *Adrβ2R* inhibits CREB phosphorylation and positively affects ATF4 phosphorylation. Consequently, the sympathetic tone favors expression of *RankL* in osteoblasts, the most powerful osteoclast differentiation factor [35, 36] (Fig. 1). Thus, the sympathetic tone altogether inhibits bone formation and favors bone resorption, in other words it reduces bone mass accrual [37, 38] (Fig. 1). This signaling mechanism is partly due to triggering of HDAC4, a histone deacetylase that binds to ATF4 in the nucleus of the cells [39]. Overall, the sympathetic tone decreases bone mass by acting through *Adrβ2R*, specifically in osteoblast.

The identification of *Adrβ2R* as the target of sympathetic regulation of osteoblasts indicated the presence of a central regulatory loop [24, 35]. The fact that bone remodeling is an asynchronous and timely regulated process suggested the involvement of the molecular clock. In testing this possibility, Fu et al. identified a series of “clock” genes as prime osteoblastic targets for negative sympathetic regulation of their proliferation by leptin [40] (Fig. 1). The clock genes encode a family of proteins that are expressed in a circadian pattern. They integrate environmental signals such as light, feeding cues and hormonal signals to regulate peripheral clock genes through neuronal signals. This happens downstream of the *Adrβ2R*-dependent activation of CREB, and in parallel to the activation of the AP1 (activator protein-1) family of transcription factors. Both AP1 and molecular clock components are up-regulated by CREB, but have opposite and exclusive effects [40, 41]. Indeed, while AP1 family members increase

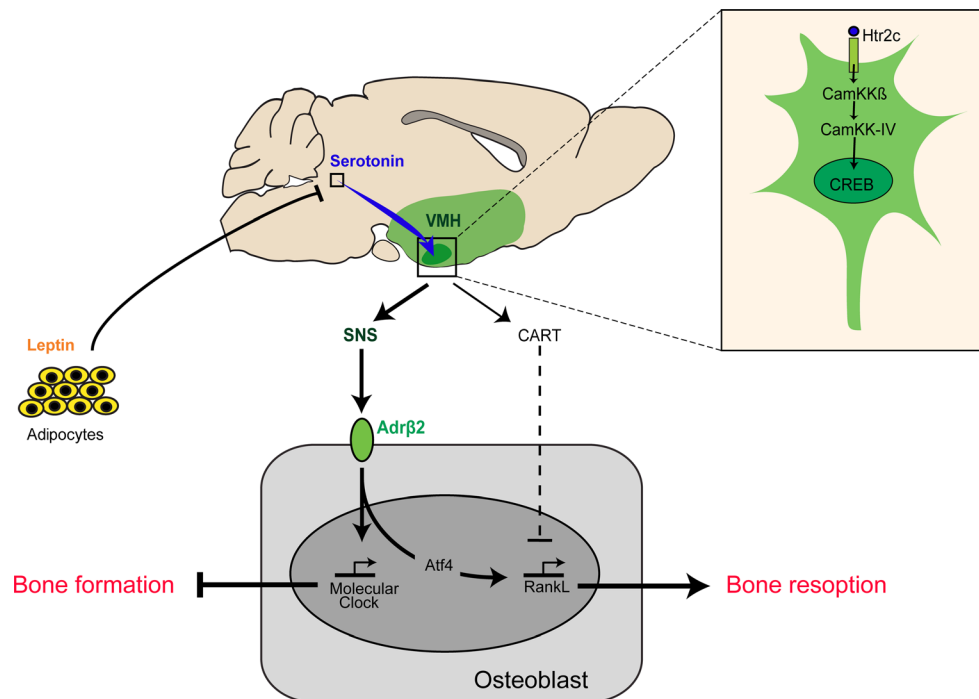


Fig. 1 Following the leptin thread: the first link between brain and bone mass. By studying the leptinergic regulation of bone mass, it was shown to act through a central relay and that serotonin is its major mediator in the brain. In the ventromedial hypothalamus (VMH), serotonin activates *Htr2c*-expressing neurons leading to a CaMKKβ – CamKK-IV – CREB cascade that is activating the sympathetic nervous system (SNS). SNS

inhibits bone formation and increases bone resorption. Through *Adrβ2* in osteoblasts, the SNS enhances *RankL* expression, the major factor of osteoclast differentiation, and recruits several components of the molecular clock to decrease bone formation. In parallel, *CART* inhibits *RankL* expression, balancing the effects of the sympathetic tone on bone mass

osteoblast proliferation through an increase in *c-myc* and *cyclin D1*, the molecular clock downregulates these two factors and, consequently, decreases osteoblast proliferation. In addition, the molecular clock prevents the activation of *AP1*, creating a theoretically antiphase pattern, in which *AP1* and the molecular clock components are exclusive in their osteoblastic function [40].

2.2 Brain-derived serotonin binds leptin to neuroskeletal physiology

In search of its mode of action in the brain, it was initially thought that the hypothalamus was the mere site of action for leptin [42]. However, this view was challenged by the landmark study by Balthasar et al., in which *ObRb* was selectively inactivated in VMH-neurons using *Sf1* (steroidogenic factor-1)-*Cre* mice, surprisingly not recapitulating the phenotype observed in *ob/ob* mice [43]. This study indicated that leptin might signal first elsewhere in the brain to favor or inhibit the synthesis of one or several neuropeptides that will consequently influence VMH neurons. Facing these results, Yadav et al. [44] performed a high-throughput analysis of neurotransmitters contents using HPLC measurement in mice lacking *ObRb* and detected a major increase of serotonin (5-HT) in the brainstem, while ICV infusion of leptin in WT mice decreased the brain serotonin content. In addition, it is now

widely accepted that patients treated with serotonin reuptake inhibitors (SSRIs) develop low bone mass, confirming serotonergic circuits as good candidates bridging leptin and the hypothalamus. Brain-derived serotonin is a neurotransmitter that influences brain development and various cognitive functions. Brain-derived serotonin is synthesized from the amino acid L-tryptophan via a short metabolic pathway that involves the rate-limiting enzyme *Tph2* (tryptophan hydroxylase 2), which is only expressed in the raphe nuclei of the brainstem and catalyzes tryptophan to 5-hydroxytryptophan (5-HT) [44]. Mice lacking *Tph2* exhibit low bone mass due to a decrease in bone formation and an increase in bone resorption. Nevertheless, the fact that leptin requires hypothalamic integrity to exert its function as well as integrity of *Sf1*-positive neurons in VMH nuclei, led Yadav et al. to search for the connection between those two anatomical systems. Axonal-tracing analyses revealed that serotonergic neurons of the brainstem project toward the VMH region of the hypothalamus. Studying the molecular pathway triggered by serotonin in the hypothalamus, it was first shown that *Htr2c* is the most abundant serotonin receptor expressed in VMH neurons and that its inactivation leads to a low bone mass phenotype. This low bone mass phenotype was correlated with an increase in the sympathetic tone in bone.

The intracellular cascade elicited by serotonergic *Htr2c* activation in VMH neurons implies *CREB* as the major

transcription factor. Transcriptional activity of CREB is regulated by the calcium-activated signaling pathway, which modulates the transcription of genes with cAMP response elements. CaMKK β and CaMKIV appeared to be the most efficient of the calmodulin-dependent protein kinases (CamK) in phosphorylating CREB. To determine to what extent this CaMK/CREB signaling mediates brain-derived serotonin regulation of bone mass accrual, cell-specific gene inactivation models were generated and analyzed. First, mice harboring a floxed allele of either CaMKK β or CaMKIV were crossed with Sf1-Cre transgenic mice that express the Cre recombinase only in Sf1-expressing neurons of the VMH nuclei. Using histological methods and micro-computed tomography, mice lacking either CaMKK β or CaMKIV in Sf1-expressing neurons displayed a severe low bone mass phenotype identical to the one seen in mice lacking brain-derived serotonin or the Htr2c receptor. Second, using compound mice, it was shown that brain-derived serotonin, via its binding to Htr2c in VMH neurons, uses a CaMK/CREB-dependent signaling cascade involving CaMKK β and CaMKIV to decrease the sympathetic tone and consequently to increase bone mass accrual [45]. This study also led to the identification of the two genes involved, encoding tyrosine hydroxylase (Th), a rate-limiting enzyme for catecholamine biosynthesis, and encoding butyrylcholinesterase (Bche), an inhibitor of acetylcholine, the main neurotransmitter of the parasympathetic nervous system. Together, these data indicate that serotonin triggers the CamK/CREB-dependent signaling cascade in VMH neurons and hereby induces a decrease of the sympathetic tone and, consequently, bone mass accrual.

In addition to leptin, it is important to mention adiponectin (Adp), another molecule secreted from adipose tissue. Adp regulates numerous metabolic processes and was recently identified as an important player of the central regulation of bone mass. By means of mice lacking Adp (*Adp*^{-/-}), it was shown that Adp had an effect opposite to leptin in the regulation of sympathetic tone and bone remodeling [46]. After crossing the blood–brain-barrier, Adp binds to neurons of the locus coeruleus and decreases the neuronal activity of Foxo1 [46]. This in turn increases the sympathetic tone and, consequently, decreases bone mass accrual by acting on osteoblasts and osteoclasts. Surprisingly, by binding to a yet unknown osteoblastic receptor, Adp also enhances bone formation through a direct peripheral action. Interestingly, during ageing, the equilibrium between peripheral and central actions of Adp on bone formation is reversed and therefore offers a promising research track for studying age-related bone decline.

2.3 Deeper insight from the hypothalamus to the bone

By first describing the role of hypothalamus and the sympathetic nervous system (SNS) in the neural regulation of bone

mass, Dr. Karsenty and colleagues laid the ground for a new understanding of skeletal physiology. Several efferent neural pathways parallel to the SNS acting on the main web of the hypothalamus–bone pathway are nowadays continuously added to this canvas.

2.3.1 Cocaine amphetamine regulated transcript

The cocaine amphetamine regulated transcript (CART) is a peptide found in the brain and in the bloodstream whose expression is regulated by leptin [47, 48]. In the hypothalamus, CART acts as a mediator of central leptin regulation of bone mass [35, 47, 48]. The absence of CART enhances bone resorption similarly to what is seen in leptin-deficient mice, suggesting a crucial role for CART in the central regulation of bone mass accrual [35]. In the periphery, CART regulates osteoblasts by decreasing *RankL* expression and bone resorption [10, 49].

2.3.2 Neuropeptide Y

Neuropeptide Y (NPY) and its receptors have emerged as regulators of bone homeostasis, regardless of leptin, influencing osteoblast and osteoclast activity. NPY is expressed both in the central and peripheral nervous systems [50, 51] and belongs to a class of peptides including pancreatic polypeptide (PP) and peptide YY (PYY). It signals through five different receptors (Y1, Y2, Y4, Y5 and Y6) and modulates several physiological processes. By acting on two of its receptors, Y1 and Y2, locally or in hypothalamic NPYergic neurons, NPY decreases osteoblastic activity and, thus, determines bone mass. This notion is supported by the demonstration of an increase of bone mass in mice lacking Y1 or Y2 receptor genes. While the Y1 receptor signaling is involved in the local control of bone turnover, Y2 receptor modulates bone mass accrual through hypothalamic neurons. This was demonstrated using *Y1*^{-/-} mice, which displayed a high bone mass caused by an increase in bone formation [52, 53]. Mice with a selective inactivation of Y2 in the hypothalamus had increased cancellous and cortical bone mass as well as greater bone formation, albeit no change in bone resorption [54].

2.3.3 Neuromedin U

Neuromedin U (NMU) is a neuropeptide that is expressed in nerve cells in the peripheral nervous system, e.g. in the submucosal and myenteric plexuses of the intestine as well as in the central nervous system, including brain regions such as the dorsomedial nucleus of the hypothalamus. NMU acts on appetite, stress response and SNS activation [55, 56] and exerts a negative control of bone mass via its binding to NMU receptor 2 (NMUR2) in the hypothalamus, regardless of the central action of leptin. Therefore,

NMU^{-/-} mice exhibit a high bone mass with increased bone formation, yet a normal proliferation and differentiation of osteoblast [57]. This observation suggests that NMU acts centrally and in parallel to leptin or SNS by binding to the NMUR2 receptor in osteoblasts. This allows for control over components of the osteoblast molecular clock, thus modulating the negative arm of sympathetic regulation and hereby counterbalancing the overall action of leptin on bone. This was confirmed in double heterozygous mice for *AdRβ2* and *NMU*, showing a concerted action of those two pathways in osteoblast, but not centrally. Also human single nucleotide polymorphism (SNP) studies in a large population for genes encoding NU and AdRβ2 showed the exact same phenotype in bone modeling in human patients as described in mice [56].

2.4 The parasympathetic nervous system regulates bone mass centrally and peripherally

In most organs, the sympathetic tone is counterbalanced by the parasympathetic activity. These two branches of the autonomic nervous system act in balance through distinct anatomical routes. The parasympathetic nervous system (PNS) functions are mediated by acetylcholine (ACh) [58]. In the central nervous system, ACh is released by the preganglionic neurons and stimulates the postganglionic neurons in the periphery by binding to the nicotinic ACh receptor (nAChR). At the postganglionic level, ACh activates the target organ by binding to the muscarinic ACh receptor, a metabotropic G-protein coupled receptor, of which five have been described so far (M1R to M5R) [59].

The demonstration of the major sympathetic regulation of bone mass prompted the question whether the PNS would counterbalance bone mass regulation of the SNS. Deletion of *M3R* in neurons, but not *M1R*, *M2R* or *M4R*, resulted in low bone mass, independently of endocrine signals [59]. Furthermore, direct parasympathetic innervation was shown by positive staining of cholinergic neurons in bone [60, 61]. Interestingly, *M3R* in osteoblasts is very poorly expressed in contrast to high expression levels in the brain in the locus coeruleus and dorsal raphe. In contrast, $\alpha2$ and $\beta2$ subunits of nAChR are highly expressed in osteoblasts and osteoclasts. Using cholinergic agonists and $\alpha2$ -nAChR^{-/-} mice, it was shown that cholinergic signaling on nAChR inhibits bone resorption that follows osteoclast apoptosis and osteoblast proliferation [61]. Furthermore, *M3R*^{-/-} mice show a low bone mass phenotype, which is associated with low sympathetic tone. In contrast, *Adrβ2*^{+/-};*M3R*^{+/-} compound mice have normal bone mass [59], suggesting a model of osteogenic activity by the PNS through the central inhibition of the SNS and peripheral innervation of nAChR in osteoblasts and osteoclasts (Fig. 2).

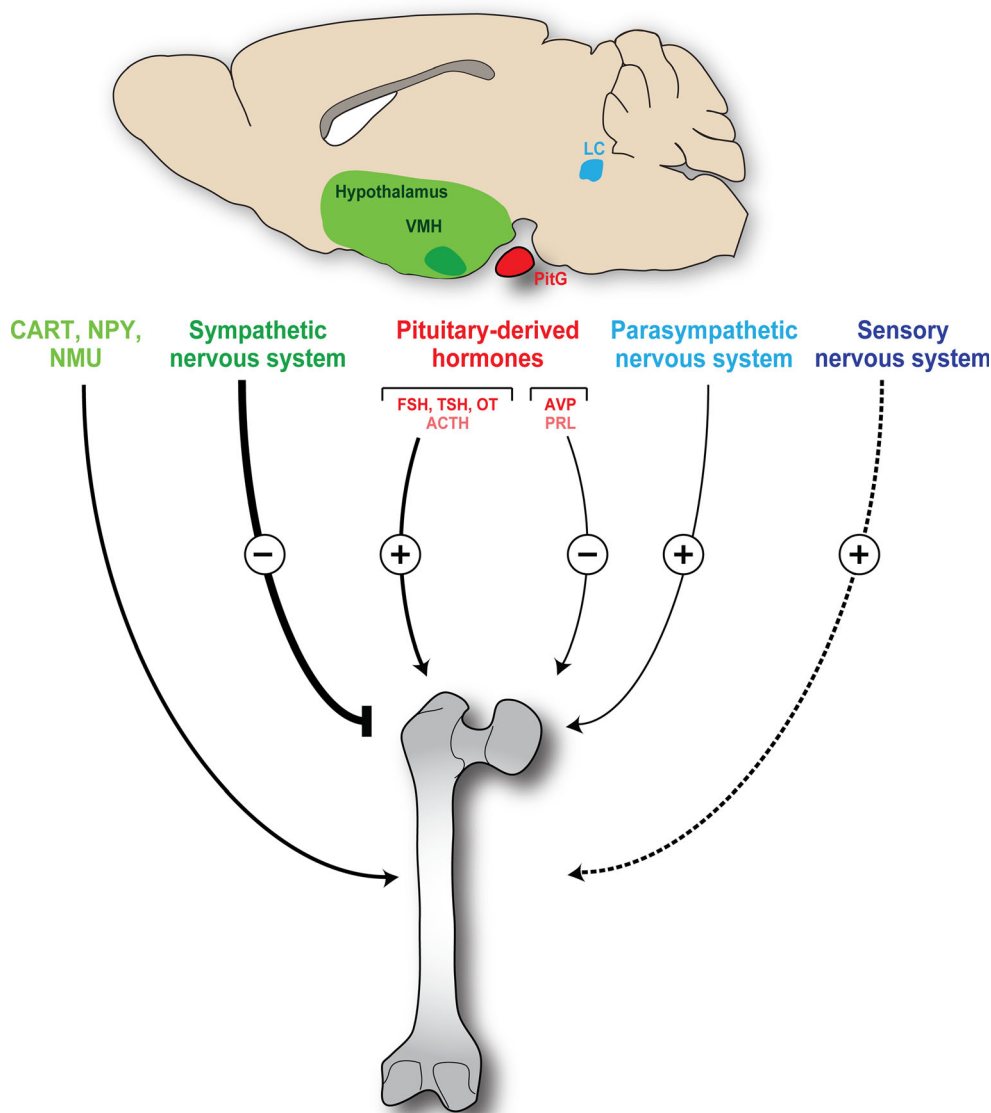
2.5 Sensory nervous system: an emerging bone regulator, the semaphorins

The demonstration that components of the autonomic nervous system tightly regulate bone mass raised the genuine question whether other types of neural systems might regulate bone turnover in vertebrates. Exploring this question, recent studies have shown that sensory afferents might positively regulate bone mass by counterbalancing the powerful effect of the SNS. When searching for candidates that hamper osteoclast differentiation, semaphorin 3A (Sema3A) was identified as a powerful osteoprotector secreted by the osteoblast [62]. Sema3A belongs to a family of axonal guidance cues, which serve as chemo-repellents when expressed in the early nervous system [63]. Interestingly, *Sema3A*^{-/-} mice exhibit a low bone mass phenotype, resulting from an increase of osteoclastic activity [62]. This feature was confirmed in culture, where osteoclast and osteoblast differentiation was prevented and increased, respectively, after Sema3A treatment [62]. The fact that Sema3A is expressed specifically in osteoblasts suggested an elegant model whereby an osteoblast-derived protein would act in a bimodal fashion by targeting its receptor, neuropilin-1 (Nrp1), in osteoblast and, through canonical Wnt and PLC γ signaling, in osteoclast [62]. Further *in vitro* data suggested that Sema3A, when expressed in osteoblast culture [64, 65], might modulate osteoclast differentiation. However, the physiological relevance of osteoblast-derived Sema3A *in vivo* still remained unclear [65]. In fact, a low bone mass phenotype was only observed after Sema3A inactivation in neurons but not in osteoblasts. Furthermore, anatomical analysis of *Sema3A*^{-/-} mice showed a specific defect in sensory innervation while sympathetic innervation remained unchanged. The fact that Sema3A preferentially acts during development may provide a basis to understand the pruning of sensory axons to their target regions in bone. Noteworthy, Sema3A has emerged only during vertebrate evolution and its partial role in bone development is thus not surprising. Moreover, studies performed in patients suggest circulating Sema3A levels as a potential predictor of bone health. In addition, other members of this gene family are implicated in skeletal features. These data add the sensory nervous system to the panel of neural pathways that influence bone physiology [65]. However, the entire spectrum of the role of semaphorins in bone health remains to be fully determined.

2.6 Straight-forward hypothalamo-pituitary-skeletal axes

All peripheral hormones regulated by the hypothalamic-pituitary axis are widely described as strong regulators of bone mass: sex steroid hormones, cortisol, IGF1 (insulin-like growth factor 1), and thyroid hormones T3 and T4. In spite of strong modulation of bone mass and a tightly regulated central control, the action of these hormones secreted in the

Fig. 2 The central nervous system influences bone physiology through the peripheral nervous system, pituitary-derived hormones and other brain-derived molecules. Emerging from the ventromedial hypothalamus (VMH), the sympathetic nervous system (SNS) down-regulates bone mass. Other hypothalamic-derived molecules that also act in bone physiology include CART, NMU, NPY. The parasympathetic nervous system (PNS) is suggested to emerge from the locus coeruleus (LC) to up-regulate bone mass. The sensory nervous system is suggested as a positive modulator of bone mass; however, the brain region mediating its function has not yet been identified. The pituitary-derived hormones TSH, FSH, oxytocin (OT) and adrenocorticotropic hormone (ACTH) increase, while prolactin (PRL) and vasopressin (AVP) decrease bone mass



periphery outreach the borders of this review and cannot be detailed here. Nevertheless, in recent years it was suggested that hormones secreted by the pituitary gland bypass their classical targets in order to act directly in the skeleton and to regulate bone remodeling.

2.6.1 Thyroid stimulating hormone (TSH)

The first and most surprising discovery of a direct regulation of bone turnover by a pituitary-derived hormone was the demonstration that TSH protects from low bone mass. In *TSHR*^{-/-} mice, osteoclastogenesis and osteoblastogenesis are up-regulated, an abnormal balance leading to high-turnover osteoporosis [66]. TSH inhibits bone formation and resorption through distinct and independent mechanisms. In osteoclast, TSH inhibits JNK and IκBα phosphorylation, decreases c-jun

and NFκB activity and prevents expression of *API* genes and *TNFα*. In osteoblast, TSH downregulates *Runx2*, *Osterix* and *Lrp5* expression, three major regulators of osteoblast differentiation and proliferation [66, 67]. This is important because it broadens the understanding of hyperthyroid-associated osteoporosis. In fact, data from hyperthyroid mice demonstrated that the low bone mass phenotype in this context is exacerbated in the absence of bone cell response to TSH [68].

2.6.2 Follicle stimulating hormone

Additional studies by the same group demonstrated that Follicle Stimulating Hormone (FSH), the master regulator of sex hormone production, targets bone cells in a straight-forward fashion and up-regulates bone mass. Mice lacking the FSH receptor (*FSHR*^{-/-} mice) had normal bone mass in the face of

gross features of hypogonadism, a condition leading to dramatic loss of bone integrity [69]. In addition, mice heterozygous for the *FSH-β* gene (*FSHβ*^{+/-} mice) with reduced FSH but normal estrogen status had increased bone mass. Confirming this mechanism *in vitro*, it was next demonstrated that FSH influences bone mass via binding to its receptor *Gi2α* in osteoclasts [69]. In addition, an elegant study performed in humans showed that the onset of osteoporosis in women at perimenopause, i.e. one year before their final menstrual period, is associated with increasing levels of FSH but normal and constant levels of serum estradiol [70]. Altogether, these data depict a mechanism whereby FSH antagonizes sex steroid action in bone, potentially providing better insight into the low bone mass observed after menopause.

2.6.3 Prolactin

Prolactin (PRL) is a small peptidic hormone secreted by the anterior pituitary gland. It is mostly known for its direct role in lactation onset after pregnancy [71]. Apart from its appealing role as a synthesis inhibitor of sex hormones, PRL was recently shown to act directly on bone. Patients with hyperprolactinemia due to prolactinoma or chronic use of dopamine-related antipsychotic drugs tend to have low bone mass [72, 73]. In addition, the PRL receptor is expressed in bone cells [74, 75]. By studying ovariectomized rats and *in vitro* osteoblasts, Seriwatanachi et al. suggested that PRL may act directly to enhance bone turnover by modulating RANKL to osteoprotegerin (OPG) ratio [74–76]. This suggests PRL as a direct regulator of bone mass, independently of prolactinemia-induced hypogonadism. Nevertheless, the clinical and fundamental body of evidence for a direct action of PRL in bone is only at its infancy and further studies are required.

2.6.4 Oxytocin

Oxytocin (OT) is a primitive mammalian neurohypophysial hormone that is produced in the paraventricular nucleus of the hypothalamus, and then stored in and secreted by the posterior pituitary gland. This hormone might also be expressed in various organs during pregnancy. Traditionally, OT is viewed as a modulator of lactation and social behavior [77]. However, it was recently demonstrated that OT acts also as a direct regulator of bone mass. Deletion of OT or the OT receptor in male or female mice causes osteoporosis resulting from reduced bone formation. OT stimulates the differentiation of osteoblasts by up-regulating *BMP-2* expression, which in turn controls *Schnurri-2* and *3*, *Osterix*, and *ATF4* expression; it furthermore stimulates osteoclast formation both directly, by activating NF-κB and MAP kinase signaling, and indirectly through the up-regulation of *RANKL* [75].

2.6.5 Arginine-vasopressin

The second hormone known to be secreted by the neurohypophysis is arginine-vasopressin (AVP). AVP, also known as the antidiuretic hormone (ADH), is a small peptide regulating water and sodium retention in the body and the constriction of blood vessels. It has been recently hypothesized that AVP might influence bone remodeling as well [78]. Given the low bone mass observed in patients with hyponatremia, i.e. low blood sodium followed by high AVP levels, Tamma et al. investigated whether AVP may act directly on bone cells. Using pharmacological, genetic and *in vitro* assays, it was shown that AVP decreases osteoblast proliferation while increasing osteoclastic activity through its receptor *Avpr1* and *Avpr2* [78].

2.7 Toward an integrated picture of neuroskeletal physiology

While we have integrated the main streams of the neural regulation of bone mass in this section, new factors have been recently identified. First, it has been shown that brain derived neurotrophic factor (BDNF) is implicated in bone turnover. Indeed, its specific inactivation in the brain resulted in a high bone mass phenotype with no change regarding sympathetic tone [79]. Second, the adrenocorticotropic hormone (ACTH) secreted by the anterior pituitary gland has proved to be an osteoprotective factor against glucocorticoid-induced osteonecrosis by increasing the vascular endothelial growth factor (VEGF) synthesis from osteoblast [80]. Since the seminal discovery that brain is a major regulator of bone physiology, a complex canvas of neuroskeletal biology is starting to be built. This growing importance of brain over bone physiology and the emerging endocrine role of the skeleton begged for the genuine and yet crucial question: can bone regulate any function of the brain?

3 Osteocalcin defines an endocrine pathway from the skeleton to the brain

The recent demonstration that bone is an endocrine organ, secreting at least two hormones, FGF23 and osteocalcin (Ocn), has advanced our understanding of several physiological processes and has enriched the physiological importance of the bone [3–6]. Through the secretion of FGF23, bone regulates phosphocalcic metabolism by coordinating the absorption and excretion of phosphate and calcium in gut and kidney. More recently, it was discovered that bone secretes a second hormone, Ocn, which does not directly act on the skeleton, but, via a feedback loop control mechanism, influences diverse powerful regulators of bone physiology.

Ocn, a 46 amino acid-long protein, synthesized and secreted by osteoblasts, is the most abundant non-collagenous

protein of the extracellular matrix of the bone that is copiously present in the circulation. Ocn has an unusual mode of activation that relies on the interplay between osteoblasts and osteoclasts. Osteoblasts produce and secrete a carboxylated, i.e. inactive form of Ocn [5, 9]. This vitamin-K dependent carboxylation of three glutamic acid residues by gamma-glutamyl carboxylase is a post-translational modification that increases the affinity of Ocn for mineral ions. Subsequently, the activity of the osteoclasts creates a resorption lacuna with a low pH (pH 4.5), which is necessary and sufficient to decarboxylate, i.e. activate, Ocn that is released in the general circulation [6, 9, 23]. Consequently, Ocn bioactivity depends on bone remodeling, rendering this hormone an authentic reflection of bone health. This feature of Ocn and the fact that it is so abundant in mineralized extracellular matrix suggested that this protein is involved in the mineralization of the extracellular bone matrix. Rather, Ocn favors pancreatic β -cell proliferation and secretion of insulin in the pancreas, insulin sensitivity in muscle and white adipose tissue, and energy expenditure [5, 9, 81]. Moreover, it also enhances testosterone biosynthesis in Leydig cells of the testis [6, 11–13]. These endocrine functions are mediated by a G-protein coupled receptor (GPCR) belonging to the calcium-sensing receptor family, Gprc6a [5, 7–9], expressed both in testis and pancreas [13].

Taking advantage of the unique fertility phenotype observed in *Ocn*^{-/-} and *Gprc6a*^{-/-} mice that phenocopy a rare but well-defined syndrome in humans, called primary testicular failure, we investigated the biological relevance of the endocrine role of osteocalcin in humans. Indeed, the genomic analysis of a cohort of patients with this syndrome identified two individuals harboring an identical loss-of-function point mutation in GPRC6A. Furthermore, through its ability to enhance bone resorption, it was shown that the reproductive function of osteocalcin is regulated by insulin signaling in osteoblasts. It was furthermore demonstrated that mice lacking Gprc6a failed to respond to Ocn *in vivo*, thus formally confirming Gprc6a as a receptor for Ocn signals [6, 82]. Further studies have confirmed these experiments *in vivo* [82] and *in vitro* [84] and extended the role of this receptor to humans [11].

3.1 Osteocalcin influences behavioral functions in adult mice

In the past 15 years, a combination of mouse and human genetic studies, in addition to molecular and cell biological assays, have identified the brain as a powerful albeit negative regulator of bone growth via the sympathetic nervous system [22–24]. This observation raised the following paramount question: how does bone growth ever occur in the face of such powerful negative influence? Since bone, following a feedback loop control, affects multiple physiological functions, it was hypothesized that bone should signal back to the brain in

order to modulate the extent of bone growth inhibition and that, hereby, bone might influence behavioral and cognitive features. Clinical studies and experimental evidence reinforced the need to investigate the specific role of Ocn in this potential crosstalk between bone and brain. First, several skeletal dysplasias affecting bone formation are associated with cognitive impairment. This is true in particular for patients harboring cleidocranial dysplasia, a disease caused by haploinsufficiency of *RUNX2* [85], an osteoblastic-specific transcription factor described as the master regulator of Ocn expression. Second, Oury et al. observed a marked passivity of *Ocn*^{-/-} mice compared to WT littermates, a behavioral feature that was first confirmed by decreased locomotor activity in the home-cage during both light and dark phases as well as in an open-field paradigm [25]. These initial behavioral findings prompted further, detailed investigation of the crosstalk between bone and brain.

3.1.1 Ocn affects several neurotransmitters and behaviors

To determine the origin of this marked passivity of the *Ocn*^{-/-} mice, a battery of behavioral tests was performed in *Ocn*^{-/-} mice, which revealed increased anxiety- and depression-like behaviors, coupled with a decrease in memory function of *Ocn*^{-/-} mice compared to WT littermates [25]. This behavioral phenotype was explained, at least in part, by changes in several neurotransmitter levels in adult *Ocn*^{-/-} brains, including a significant decrease of the three monoamine neurotransmitters norepinephrine, serotonin and dopamine as well as a significant increase in GABA content [25]. Of note, the decrease in norepinephrine content observed in the brains of *Ocn*^{-/-} mice may explain the high bone phenotype originally noted in this mouse model [86]. Consistently, it was next shown that the expression levels of *Tph2*, a gene encoding the initial enzyme required for brain serotonin synthesis [86], and *Th*, encoding the initial enzyme in dopamine and norepinephrine synthesis, were decreased in the *Ocn*^{-/-} brain. Conversely, expression of *Gad1* and *Gad2*, two enzymes required for GABA biosynthesis, were increased in *Ocn*^{-/-} mice. Importantly, behaviorally and regarding neurotransmitter levels, the *Gprc6a*^{-/-} mice were indistinguishable from WT littermates. This makes it unlikely that this receptor transduces Ocn signaling in the brain. This last notion is also important because it implies that the decrease in activity observed in *Ocn*^{-/-} mice is not a mere consequence of metabolic or reproductive abnormalities, since these are equally severe in *Ocn*^{-/-} and *Gprc6a*^{-/-} mice [6, 12, 13, 84].

3.1.2 Ocn signals in the brain

Several observations indicate that bone-derived Ocn, which is not expressed in any part of the brain neither during developmental nor adult stages, regulates behavioral functions. Ocn,

when uncarboxylated, is able to cross the brain–blood-barrier and binds specifically to neurons in several parts of the brain such as the hippocampus, the ventral tegmental area, substantia nigra and the raphe nuclei of the brainstem. Treatment of brainstem and midbrain explants with Ocn led to an increase in *Tph2* and *Th* expression, while decreasing *Gad1* and *Gad2* expression. Treatment of hindbrain neurons with Ocn affects calcium flux and the action-potential-firing rate of various neurons and interneurons of the brainstem. Lastly, GABAergic interneurons of the brainstem showed decreased firing rates while firing rates in neurons of the dorsal raphe were enhanced by Ocn treatment [25].

3.1.3 Postnatally, Ocn regulates anxiety-, and depression- and memory-like behaviors

The evidence that Ocn acts as a neuro-active molecule in the central nervous system triggered the immediate question whether Ocn influences behaviors and neurotransmitters, postnatally and/or during embryogenesis. This fundamental question was addressed by engineering mice with a time-dependent inactivation of *Ocn* exclusively in osteoblasts, i.e. mice harboring a floxed allele of *Ocn* [5] were crossed with mice expressing *Cre^{ERT2}* under the control of the osteoblast-specific regulatory elements of *Coll1a1* [33]. Tamoxifen-treated *Ocn_{osb}^{ERT2}* mice showed a significant increase in anxiety-like and depression-like behaviors compared to controls. In stark contrast, spatial learning and memory were only modestly affected in tamoxifen-treated *Ocn_{osb}^{ERT2}* mice. Subsequently, when infusing Ocn intracerebro-ventricularly (ICV) in the *Ocn*^{−/−} brain, these anxiety- and depression-like phenotypes were rescued, while the spatial learning and memory defect, which was also observed in *Ocn*^{−/−} mice, was only partially reversed. Taken together, these observations suggest that Ocn may influence memory-like behavior through developmental mechanisms during embryogenesis, while the anxiety- and depression-like phenotypes were inducible in the adult brain.

Exploring the postnatal effect of this bone-derived hormone, it was furthermore shown that Ocn influences adult hippocampal neurogenesis (AHN) in the dentate gyrus, one of the restricted brain regions that, throughout lifetime, generates functional neurons from adult neuronal precursors. AHN is dynamically regulated by intrinsic as well as extrinsic cellular mechanisms, involving diverse signaling pathways and molecular players, such as neurotransmitters, transcription factors and epigenetic regulators [88]. The link between Ocn and age-related cognitive decline might include a decrease in bone mass as well as a decline in glucose homeostasis, fertility and cognitive functions, three physiological processes regulated by Ocn. Hence, it suggests that Ocn might be required to prevent organismal aging by maintaining various physiological functions at their optimal level.

3.2 Osteocalcin is a nurturing factor for fetal brain development

The partial deficit in learning and memory observed in adult, tamoxifen-treated *Ocn_{osb}^{ERT2}* mice and the incomplete rescue of this defect after ICV injections of osteocalcin suggested that Ocn may shape memory functions during embryogenesis. Interestingly, while endogenous *Ocn* expression was not detected in the developing skeleton of the mouse embryo until E16.5, the protein was already detectable in the blood of E14.5 embryos, suggesting that maternal Ocn reaches the fetal blood stream (Fig. 3). The placental transport, starting at E14.5, was confirmed using an *ex vivo* dual perfusion system that monitors the transport of substances across the mouse placenta. Accordingly, Ocn was also detectable in the serum of *Ocn*^{−/−} embryos carried by *Ocn*^{+/+} mothers. To test the influence of maternal Ocn on fetal brain development and the behavior of the adult offspring, *Ocn*^{−/−} embryos carried by *Ocn*^{−/−} mothers, which, during pregnancy, were either injected daily with Ocn or remained untreated, were compared. Only *Ocn*^{−/−} embryos from *Ocn*^{−/−} mothers had an enlargement of the lateral ventricles of the brain and an

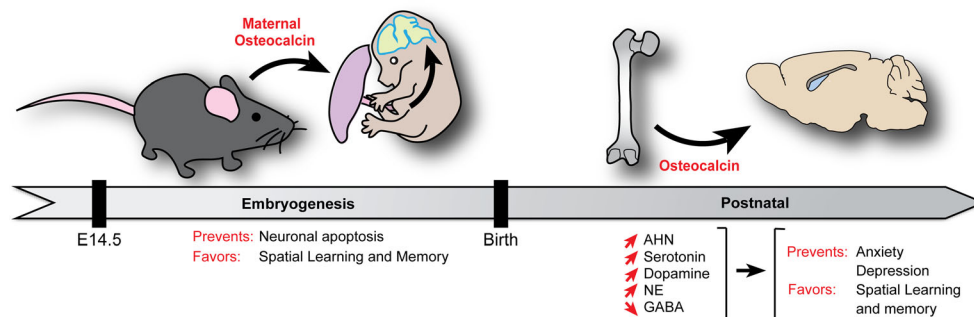


Fig. 3 Embryonic and postnatal influence of Ocn on brain. Mother-derived Ocn crosses the placental barrier from E14.5 until birth and influences brain development by preventing neuronal apoptosis and by favoring spatial learning and memory of the progeny. Postnatally, bone-derived Ocn crosses the blood–brain-barrier and influences the

production of several neurotransmitters: serotonin, dopamine, norepinephrine and GABA but also enhances adult hippocampal neurogenesis (AHN). Perhaps as a result of this, Ocn prevents anxiety- and depression-, and favors memory-related behavior

increase of apoptotic cells in the hippocampus at age E18.5. These histological phenotypes were corrected by means of daily Ocn injections during pregnancy. While this treatment only had a modest beneficial effect on the anxiety phenotype of the *Ocn*^{-/-} mice, it fully rescued their deficit in learning and memory, indicating that the learning and memory phenotype is, at least in part, of developmental and maternal origin. Taken together, these results suggest an important contribution of bone to the maternal influence on fetal brain development and cognitive health of the offspring [25].

4 Skeletal and neuropsychiatric diseases

The field of integrative physiology emerges in close interaction with clinical observations. The growing importance of bone physiology exemplifies this intimate link well. Retrospectively, several clinical observations suggest a link between bone physiopathology and brain disorders. The discovery of major determinants of osteoblastic and osteoclastic differentiation was made through clinical studies of rare diseases, such as Cleidocranial Dysplasia and Coffin-Lowry syndrome. Studying correlative clinical reports might be of great use to identify players of the intimate connection between bone and brain and in widespread diseases such as osteoporosis, algoneurodystrophy or intellectual disabilities. In this section, we propose to give a brief overview of clinical studies adding supportive evidence of a bone-brain crosstalk. Nevertheless, we have to mention here that this section is only based on correlative studies and that the potential role of the bone in neuropsychiatric disorders nowadays needs to be directly explored.

4.1 Genetic diseases of the skeleton linked to mental deficits

4.1.1 Coffin-Lowry syndrome

Coffin-Lowry Syndrome (CLS) is a rare X-linked mental retardation associated with skeletal abnormalities. CLS is caused by various loss-of-function mutations in the *hRSK2* gene [89] encoding RSK2, a growth-factor-regulated protein kinase that phosphorylates ATF4, a major osteoblast differentiation factor [89, 91, 92, 93]. In CLS patients, growth is severely delayed and digit abnormalities as well as facial dysmorphism characterize the physical appearance [90]. Mental retardation is a characteristic feature of this disease: the IQ ranges from 15 to 60, psychomotor development is retarded and the behavioral pattern of these patients is described as cheerful, easy-going and friendly [90]. Interestingly, ATF4, also known as CREB-2, is required for the induction of long-term potentiation, a form of synaptic plasticity that is crucial for memory formation [94]. In addition, ATF4 phosphorylation is required in bone for osteoblast differentiation, in turn increasing Ocn production [91, 92]. Consequently,

based on the recent disclosure of bone regulation of memory and the mental retardation observed in CLS, it is not elusive to hypothesize a link between the mental deficits observed in CLS and the disruption of osteoblastic differentiation due to a decline in ATF4 activity.

4.1.2 Cleidocranial dysplasia

The first determinant of osteoblast differentiation, RUNX2, was discovered through genetic studies in patients with cleidocranial dysplasia (CCD). Indeed, this disease is mainly caused by heterozygous mutations in the *RUNX2* gene (also known as *OSF2/CBFA1*) [85, 95]. This haplo-insufficiency of RUNX2 leads to hypoplasia or aplasia of the clavicles, delayed fusion of cranial sutures and a wide spectrum of dental anomalies [96]. In addition, some of the patients harboring CCD may also develop cognitive disorders due to poorly understood mechanisms [97]. This is consistent with the influence of the bone-derived hormone Ocn in brain development and cognitive functions. In fact, Runx2 is one of two principal and specific osteoblastic transcription factors, and in turn regulates expression of Ocn [95]. However, while bridging the endocrine link between bone defects and cognitive decline in these patients is tempting, this has to be assessed further by clinical and animal studies.

4.2 Complex regional pain syndrome

The complex regional pain syndrome (CRPS), previously known as algoneurodystrophy, is a multifactorial disorder of complex pathogenesis affecting one or several joints and the surrounding bones. The main characteristics of this syndrome are continuous and dreadful pain, joint stiffness and vasomotor disturbances. In addition, CRPS is accompanied by a rapid demineralization of bone and often the emergence of psychiatric diseases such as depression, anxiety and personality disorders [98]. The pathophysiology of CRPS is complex, involving inflammatory factors as well as the sympathetic and sensory nervous systems both in the periphery and in the brain [98, 99]. Although the involved mechanisms remain unclear, it may be of significant value to address the osteoarticular defects with regard to the psychological consequences of this disease. In addition, the aforementioned action of the nervous system on skeletal physiology may be of great use to understand this syndrome and contribute to the development of the unprecedented mechanism-based therapies [98].

4.3 Neuropsychiatric diseases and bone mass defect

4.3.1 Major depressive disorder

Major depressive disorder (MDD) is among the most prevalent diseases worldwide [100]. It is characterized by abnormal

states of bereavement and disproportionate and persistent sadness in the absence of manic episodes, i.e. hyperactivity, euphoria and increased seeking of pleasure. In practice, patients experience crying, suicidal thoughts, hedonic loss, slowness of action and speech, as well as physiological changes such as disturbances of sleep, appetite, sexual desire and transit [101]. This disease remains a major challenge of public health because of its high frequency and inconsistent response to available treatments [100]. In short, it was also shown that antidepressant drugs such as selective serotonin re-uptake inhibitors (SSRIs) decrease bone mass and increase the risk of fractures, consistent with the role of brain-derived serotonin on bone physiology [102, 103, 104].

Two main theories prevail to understand the basis of this disorder: the first theory relies on alterations of the hypothalamic-pituitary-cortisol axis leading to higher blood cortisol and enhanced stress-responses. The second one states that MDD could be due to a deficit in brain monoamines serotonin and norepinephrine [101]. Interestingly, clinical studies correlating features of bone metabolism in women with MDD indicated that depressed women had lower bone mass and lower levels of Ocn compared to healthy subjects [105]. These findings were confirmed for premenopausal women [106]. Consistent with these results, additional studies demonstrated a prevalent low bone mass density even at early stages of MDD excluding potential drug-induced bone loss. In fact, only a minority of studies failed to demonstrate low bone density in these patients. Combined with evidence from mice and the conservation of Ocn in reproductive and metabolic function in humans, these data provide an appealing ground for considering the skeleton as potent determinant of depression in human patients.

4.3.2 Schizophrenia

Schizophrenia is a major burden on patients' health. After depression, it is one of the most prevalent psychiatric disorders affecting approximately 1 % of the general population. Schizophrenia develops progressively after adolescence and early adulthood. The symptomatology generally appears sequentially, the first symptoms being cognitive and social impairment, followed by anxiety and depression, and eventually prodromal symptoms leading to psychosis [107]. Psychosis includes hallucinations, delusion, prediction errors, apathy and poor self-care, making the patients incapable of any social activity. Two theories explaining schizophrenia are currently under investigation, suggesting both a disruption of the dopaminergic system and neurodevelopmental defects, including enlargement of the ventricles and grey and white matter disruptions [107].

Interestingly, various reports have elucidated a potential association between schizophrenia and low bone mass [108]. Surprisingly and in contrast to what was observed in depressive patients, a higher concentration of serum Ocn was

reported for some schizophrenic patients [108]. Given similar behaviors and abnormalities seen in OCN^{-/-} and schizophrenic patients, this incites to investigate more seriously the relation between bone and schizophrenia.

4.3.3 Cognitive decline

Along with the ageing of populations, dementia, already affecting millions of people, is eager to accelerate. The fact that this spectrum of disorders is irreversible and that no effective therapy exists at the moment urges the understanding and therapeutic management of these disorders [109]. Particular to our interest, the age-related cognitive decline and tendency to dementia has been negatively correlated with bone mass density. Indeed, a few studies have assessed whether low bone mass can serve as a predictor of dementia onset [110, 111]. In two large population studies, it was found that people in the lowest quartile of bone density had a 2-fold increased risk of dementia, adding support to the notion that disruption of bone is associated to cognitive and ageing processes [110, 111].

4.3.4 Neurological fetopathy following use of anti-vitamin K

Lastly, concerning the maternal role of Ocn during fetal brain development, it should be noted that drugs affecting Ocn metabolism, i.e. anti-vitamin K drugs, are sources of brain development abnormalities, thus contraindicating their use during pregnancy. As Ocn metabolism clearly implicates bone turnover and vitamin K-dependant γ -carboxylation, these drugs are potential disruptors of Ocn endocrine functions. Given the recent demonstration of mother-derived Ocn in brain development in mice, the observed brain defects following anti-vitamin K treatment is striking. Indeed, prenatal exposure can lead to abnormal development of the skeleton and to mental retardation due to hydrocephalus, microcephaly and agenesis of brain structures including cortical regions [112, 113].

5 Conclusion

The seminal discovery of the central regulation of skeletal homeostasis by leptin [22] has opened the door to the exploration of an intimate connection between bone and brain. Since this first demonstration, a large spectrum of molecular players has emerged over the last 15 years, increasing considerably the importance of the central control in the regulation of bone mass. More recently, the demonstration that bone, according to a feedback loop control mechanism, may return the favor to the brain has demonstrated not only a connection but a dialogue of functional relevance between these two organs. Through the secretion of Ocn, the skeleton can influence gene expression in neurons, the synthesis of neurotransmitters and several behaviors, which is a sharp departure from the

classical view of the regulation of brain development and cognitive function. In addition, the multitude of physiological functions affected by Ocn is eager to decline with aging such as glucose metabolism, reproductive functions and cognition. Hence, a vicious cycle involving Ocn and aging may exist and this molecule might be required to maintain various physiological functions at their optimal level to prevent aging. Indeed, it suggests that bones may be a determinant as much as a victim of the ageing process. Consistently, this totally novel paradigm in the field of cognition might be enlarged to other hormonal signals that are rejuvenating the premises of a new and exciting field of neurobiology [20, 114] and confirming the growing importance of hormonal signals in the regulation of brain development and functions.

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