Chapter

Vitamins D and B_{12}, Altered Synaptic Plasticity and Extracellular Matrix

Marcela Bermudez Echeverry, Silvia Honda Takada, Bruna Petrucelli Arruda, Debora Sterzeck Cardoso, Pamela Pinheiro Martins, Juliane Midori Ikebara, Aline V. Sousa-Santos and Victor R.C. Torres da Silva

Abstract

Brain plasticity is regulated through dynamic interactions between perineuronal nets, matrix metalloproteases (MMPs) and the extracellular matrix (ECM). Several studies have identified a crucial role for vitamins D and B_{12} in brain development and a deficiency in these vitamins may contribute to the emergence of cognitive deficits, as well as the onset of both autism spectrum disorder and schizophrenia. However, the mechanisms underlying the interplay between ECM, MMPs, vitamins and these neuropsychiatric conditions are poorly understood. In this chapter, we seek to understand how the risk of neurodegeneration in vulnerable individuals and the aetiology of specific neuropsychiatric disorders are affected by vitamin D and B_{12} deficiency, in conjunction with low levels of the antioxidant glutathione, impaired GABAergic inhibition, and alterations in the permanent ECM.

Keywords: vitamin deficiency, perineuronal nets, matrix metalloproteases, parvalbumin interneurons, GABA, neurodevelopment

1. Introduction

A proteoglycan-rich matrix, the perineuronal net (PNN) is a dense structure within the extracellular matrix (ECM), whose synapses form through gaps around many neuronal bodies and dendrites at a late stage in brain development. PNNs are formed at the end of a critical period of neurodevelopment, following the transformation of the central nervous system (CNS) from an environment conducive to neuronal growth and motility to one that is more restrictive, in response to several sensory inputs from both neurons and glia driving increased neuroplasticity [1]. The main components of the PNN matrix include several chondroitin sulfate proteoglycans (CSPGs), such as hyaluronan, link proteins, and tenascin-R and -C.

During mammalian development, hyaluronan binds to members of the lectican family originally produced in neurons, including versican V0 and V1 and neurocan [2], whereas aggrecan seems to be expressed by astroglial cells in the juvenile matrix [3]. Other lecticans include versican 2, brevican, phosphacan, tenascin-R and the

link proteins HAPLN2/Bral1 and HAPLN4/Bral2 are only observed in more mature matrix environments approximately 2 weeks after birth [4–6], in contrast to the composition of the juvenile matrix. Following this period, shifts in brevican expression occur at the end of myelination, leading to white-matter precursor changes from an oligodendroglial to an astrocytic lineage [7], and resulting in a compact extracellular matrix forming the PPN [8].

PNNs have been observed 2–5 weeks after birth around parvalbumin (PV^+)-expressing GABAergic interneurons in pyramidal cortex, and around large motor neurons of the brainstem and spinal cord. This period coincides with the end of experience-dependent refinement of the synaptic network [8], but marked by a still critical period of matrix turnover and proteoglycan degradation by ADAMTS metalloendopeptidases and matrix metalloproteinases (MMPs) [8, 9]. PNN formations can also be observed in several distinct areas of the CNS, such as other regions of the cerebral cortex, the hippocampus (HPC), thalamus, and cerebellum [8].

PNNs in the adult CNS secrete hyaluronan through the action of membrane-bound HA synthase, an enzyme linked to the action of link proteins, lecticans, tenascin-R and chondroitin sulphate proteoglycans (CSPGs), creating supramolecular aggregates on the surface of neurons [1]. Other relevant glycoproteins besides CSPGs include Reelin, mainly secreted by Cajal–Retzius cells and involved in the control of neuronal migration and the establishment of cell aggregation and dendrite formation during the embryonic and early postnatal stages of development [10]. In adulthood, Reelin signalling is involved in the modulation of synaptic function and binds to very-low-density lipoprotein receptors and apolipoprotein E receptor 2 [11]. Increased clustering of Reelin receptors leads to a build-up of DAB1 proteins on the neuron membrane, greater activation of Src/SFK family kinases, and tyrosine phosphorylation of N-methyl-D-aspartate receptors (NMDARs), resulting in a net increase of receptor activity (**Figure 1**) [12]. Reelin insufficiency may lead to alterations in NMDAR clustering and LTP, such as in dysfunctional GABA-ergic transmission in the cerebral cortex and hippocampus observed among the morphofunctional signalling changes in schizophrenia (SZ) [12].

In fact, alterations of GABAergic signalling within a prenatal stress period have been identified as important factors in the development of SZ [13], autism spectrum disorder (ASD) [14], and epilepsy [15], often leading to an altered density of GABAergic cells and aberrant oscillatory activity. However, one functional model of brain development has proposed that prenatal stress involves DNA methylation, possibly inducing methylation of the gene responsible for Reelin promoter, with the consequent down-expression of Reelin resulting in abnormalities within the neuronal architecture of the prefrontal cortex, a reduction in dendritic complexity and a decreased number of GABAergic neurons, leading to altered developmental neuronal connectivity [13].

Animal studies have demonstrated that DNA methylation in the BDNF gene controls its expression during forebrain development in mice [16]. Furthermore, binding of BDNF and nerve growth factor (NGF) neurotrophins to their respective receptors (TrkB/A) triggers the PI-3kinase/AKT pathway, with activation of the mammalian target of rapamycin (mTOR) [17] and Akt-dependent inhibition of the serine/threonine kinase Gsk3β, resulting in decreased transcription of pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6) [18]. Since Reelin/lipoprotein receptors do not contain a cytoplasmic kinase domain, the core Reelin signalling pathway seems to be associated with tyrosine kinase receptor (RTK or Trk) activity [19], the most likely coreceptor candidate [20]. As such, Reelin signalling from the ECM in collaboration with TrkB/A receptor activation, leads to increased phosphorylation of

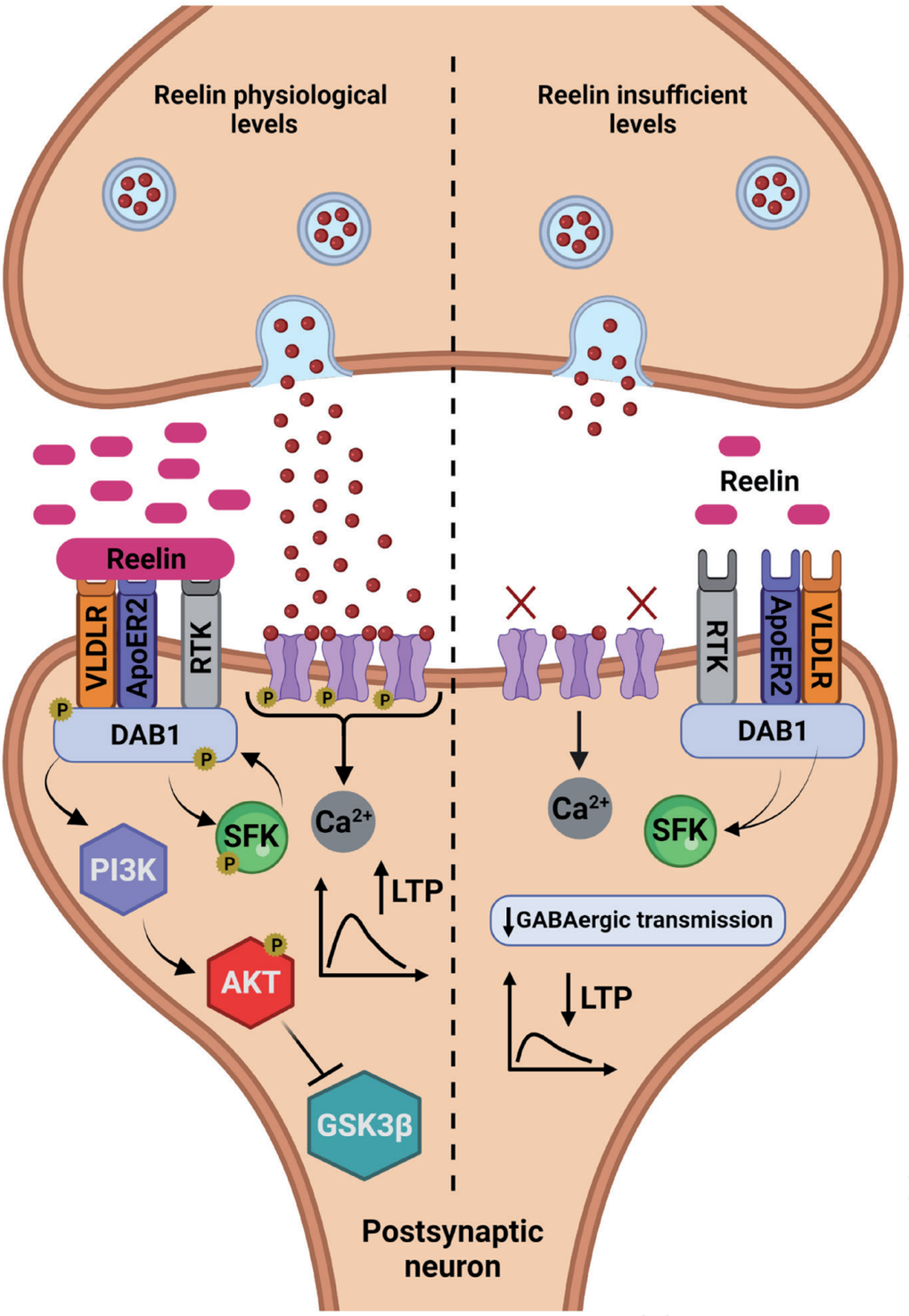

Figure 1.
Effect of Reelin concentrations in the ECM on NMDA signalling. Reelin activates adaptor protein disabled 1 (DAB1) by binding with its very-low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor type 2 (APOER2). DAB1 is phosphorylated by Src family kinases (SFK) at different sites on the protein - this phosphorylation occurs mainly through the action of a co-receptor, tyrosine kinase receptor (RTK or Trk), implicated in a variety of cellular processes including growth, differentiation, and regulation of energy metabolism in the neuron. DAB1 phosphorylation leads to inhibition of the serine/threonine kinase Gsk3β via protein kinase B (Akt), where a decrease in AKT phosphorylation levels with subsequent high levels of GSK-3β phosphorylation, has been observed in lymphocytes and brains of individuals with schizophrenia (SZ). Clustering of Reelin receptors via SKF activation also leads to greater tyrosine phosphorylation of N-methyl-D-aspartate receptors (NMDARs), resulting in a net increase of receptor activity following the induction of long-term potentiation via $Ca2^{+}$ regulation. This signalling cascade appears to be an essential process for neurobiological regulation during neurodevelopment (modified from [12]). Created with BioRender.

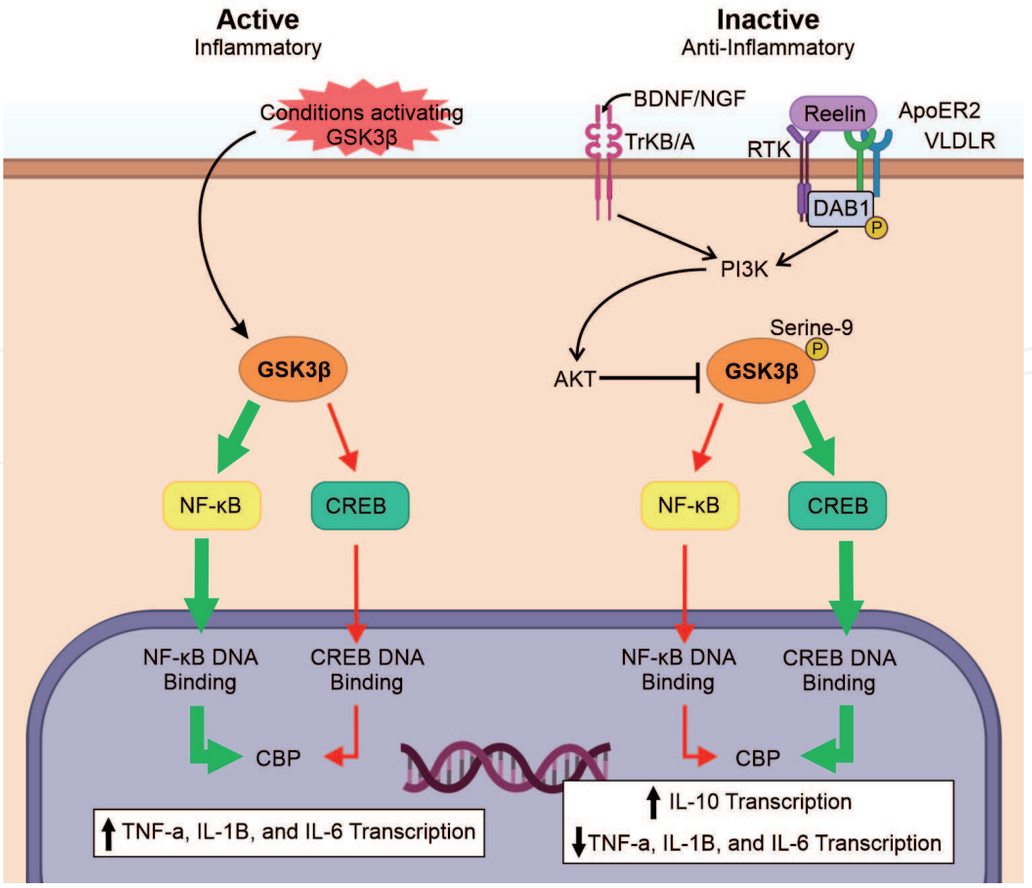

Figure 2.
*Hypothetical signalling cascades for GSK3β modulation of the expression of pro-inflammatory and anti-inflammatory cytokines in glial cells. Receptor crosstalk between receptors TrkB/A and Reelin in the ECM (see **Figure 1**), increase the phosphorylation of serine-9 GSK3β leading to GSK3β inhibition, an increase in the translocation of CREB from the cytoplasm to the nucleus, and an increase in the transcription of anti-inflammatory cytokine (IL-10). In fact, GSK3β can modulate the expression of both pro-inflammatory and anti-inflammatory cytokines. GSK3β activation in glial cells triggers the NF-κB pathway and the translocation of NF-κB from the cytoplasm to the nucleus, leading to an increase in the transcription of pro-inflammatory cytokines ((IL-1β, TNF-α, and IL-6), via the action of CREB-binding protein (CBP). Inhibition of GSK3β results in an increase of CREB translocation from the cytoplasm to the nucleus. Phosphorylated CREB binds specifically to the nuclear CBPs at transcriptional sites, resulting in increased transcription of anti-inflammatory cytokines such as IL-10 (modified from [21]).*

serine-9 GSK3β, with the inhibition of GSK3β in glial cells and leukocytes resulting in more CREB being translocated from the cytoplasm to the nucleus, and an increase in the transcription of anti-inflammatory cytokines (IL-10) (**Figure 2**) [21].

PNN development and the maturation of PV^+ inhibitory cells, as well as processes such as myelination, mark the end of the critical period of human neurodevelopment [22]. Disruption or delay to the formation of the PNN results in the resumption or extension of the time window for neuroplasticity in the brain [23], wherein the nervous system is more sensitive to epigenetic, physical, biochemical, environmental, and nutritional factors. The effects of nutrition on individuals during gestational and early development have been extensively researched, leading many researchers to conclude that nutritional factors such as vitamins, folate and iodine can cause long-lasting impacts in neurodevelopment [24, 25]. As the foetus' and newborn's acquisition of vitamins like B_{12} and D, depends to a great extent on maternal diet, such research has increasingly focussed on the impact of the mothers' vitamin deficiency on their offspring's brain development during the foetal and exclusive breastfeeding stages.

S-adenosyl methionine (SAM) is a universal methyl donor for some of the main methylation reactions. Vitamin B_{12} is an important cofactor in the one-carbon cycle

and is involved in the formation of SAM. Vitamin B_{12} supplements have been shown to improve pregnancy outcomes and reduce the risk of neurodevelopmental disorders in the developing child [26]. In rats, dose-dependent vitamin B_{12} supplementation was able to maintain the levels of docosahexaenoic acid (DHA) and BDNF in the hippocampus and cortex in pups at birth, and BDNF in the hippocampus at 3 months of age [17]. In addition, the combination of omega-3 fatty acid and vitamin B_{12} administration maintained spatial memory performance in neonates [17]. Experimental evidence suggests that DHA, together with greater levels of physical exercise, increases activated forms of CREB and synapsin I, reducing oxidative stress in the hippocampus [18].

Vitamin D deficiency may also reduce the integrity of PNNs and synaptic plasticity in neuropsychiatric disorders through the modulation of MMPs. Vitamin D deficiency has been associated with vulnerability to SZ [27], as well as ASD [28] and attention deficit and hyperactivity disorder (ADHD) [29], the two most common neurodevelopmental disorders. As mentioned earlier, ADAMTS and MMPs are two families of endogenous zinc-dependent proteases, secreted as inactive proenzymes that cleave ECM components. Alterations in the genes that encode MMP-16 and MMP-9 have been observed in patients with SZ [30]. High levels of MMP-9 can support the proteolytic cleavage of ECM with permissive synaptic plasticity but also lead to abnormal aggrecan degradation, abnormal development and neural excitability [30]. Chronic stress and neurological trauma can enhance MMP-9 levels in the brain [31, 32], and consequently raise the risk of SZ. A plausible proposal has been made that vitamin D deficiency leads to PNN degradation in patients with SZ [27]. In fact, vitamin D

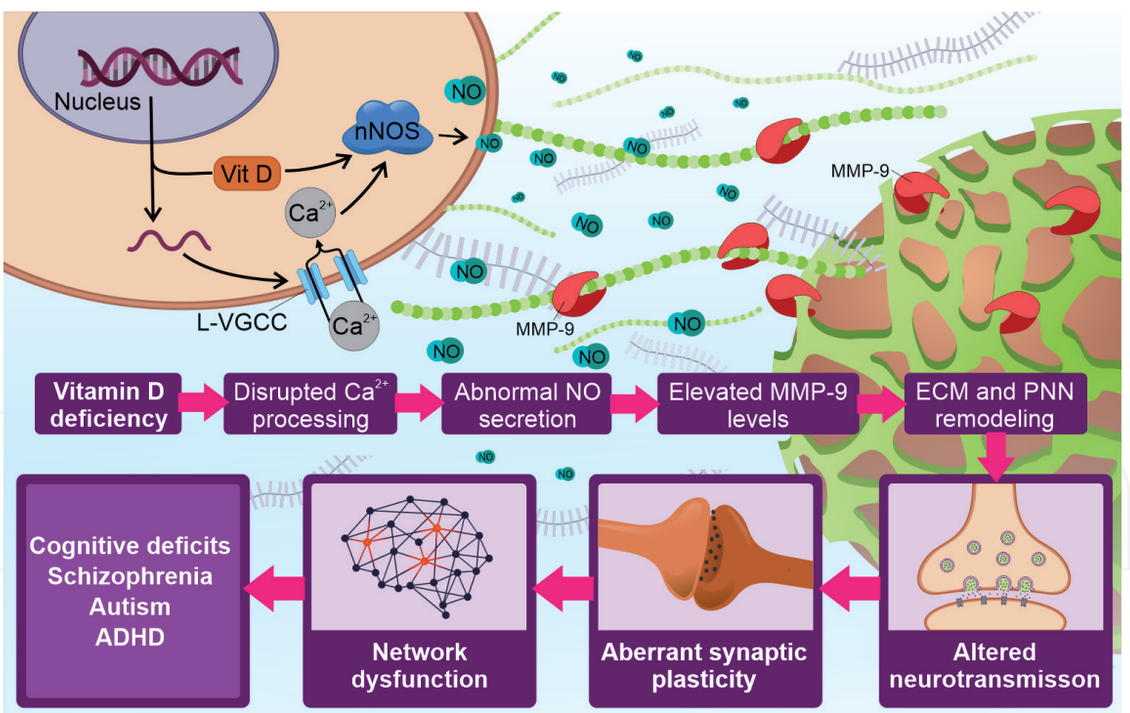

Figure 3.
Vitamin D deficiency and PNN formation during neurodevelopment. The figure above shows a neuron enveloped by a PNN. Vitamin D deficiency may induce a deficit in ECM organisation over the course of neurodevelopment, leading to the PNN loss and network-wide dysfunction in GABAergic, glutamatergic, and dopaminergic neurotransmission. Vitamin D deficiency is also linked to altered transcription of calcium channels (L-VGCC) potentially increasing the level of calcium input into the neuron, and to altered neuronal nitric oxide synthase (nNOS) activity, resulting in an increase of nitric oxide (NO) secretion into the extracellular space and elevated levels of MMP-9. This enhanced MMP-9 expression induces increased aggrecan synthesis, resulting in disruptions to the network of several neurotransmission systems important for normal cognitive function (spatial learning deficits), and SZ, autism and ADHD. Abbreviations: L-VGCC: L-type voltage-gated calcium channel; ECM: Extracellular matrix; MMP-9: Matrix metalloproteinase-9; nNOS: Neuronal nitric oxide synthase; NO: Nitric oxide; PNN: Perineuronal net; SZ: Schizophrenia; ADHD: Attention deficit and hyperactivity disorder (modified from [34]).

deficiency is associated with increased MMP-9 production [33] and calcium activity on the neuronal membrane, leading to increased nitric oxide (NO) formation and higher MMP-9 levels, and further appears to modulate its endogenous inhibitor TIMP1 (tissue inhibitor of MMP) [34]. Aggrecan-rich PNNs undergo restructuring leading to the occurrence of more synaptic anomalies and greater network dysfunction in GABAergic, glutamatergic, and dopaminergic neurotransmission, as evidenced by some forms of SZ (**Figure 3**) [34]. Cognitive deficits, such as spatial learning deficits, have been observed in adult mice with vitamin D deficiencies, with reduced density of PNNs and neural networks within the hippocampus [35].

2. Vitamin B_{12} in neurodevelopment

Vitamin B_{12} is a member of the cobalamin family and can usually be obtained in sufficient quantities from meat, eggs, and dairy products. It is critical for the production of red blood cells, and the growth and maintenance of the nervous system.

Major causes of vitamin B_{12} deficiency include autoimmune pernicious anaemia, gastrectomy, ileal resection, pancreatic insufficiency, and malabsorption syndromes [36]. The human body is incapable of endogenous B_{12} production so it must be obtained from external sources in the individual's diet [37, 38]. Currently, a nutritional B_{12} deficiency is common in vegans, a fast-growing eating trend in many Western countries [39]. Vitamin B_{12} deficiency is also common in developing countries, with more widespread occurrence over more widespread sections of society beginning in early life and persisting throughout adulthood [40].

Pregnant women require a greater amount of vitamin B_{12} (2.5 g/day) compared to the general adult population (2.4 g/day), to adequately meet the nutritional needs of the foetus via absorption through the placenta [41]. The lack of sufficient vitamin B_{12} ingestion by the mother during pregnancy results in its deficiency in breast milk and the foetal bloodstream [42, 43]. Indeed, several studies have linked maternal deficiency in vitamin B_{12} concentration to developmental complications, including spontaneous abortion [44], low birth weight [45, 46], intrauterine growth restriction [45], and neural tube defects [47]. Lack of sufficient vitamin B_{12} in the mother's diet is reflected in similarly low concentrations of B_{12} in the bloodstream of breastfed babies during the period of exclusive breastfeeding, when the baby is dependent on breast milk for vitamin absorption, despite the relatively short window of this development period [48].

As in adults, vitamin B_{12} is mainly stored in the newborn's liver and is used on demand. However, as newborns have limited hepatic reserves, even if they are born at a healthy weight and size, symptoms resulting from vitamin B_{12} deficiency may appear from as early as 2 months of age [49]. Such symptoms imply a clinical pattern including abnormal pigmentation, hypotonia, liver and spleen enlargement, anorexia and growth failure associated with poor brain growth, which were first described by Jadhav and colleagues in 1962 [50].

Cellular deficiency of vitamin B_{12} results in slower proliferation and a faster differentiation of neuroblastoma cells [51], which point to its pivotal role in cell division and differentiation. In addition, deficiency of vitamin B_{12} in rodents is associated with selective brain damage, vascular and cognitive impairment [52], long-lasting functional disabilities in exploratory behaviour, learning and memory functions, and a mild decrease in hippocampal neurogenesis [53]. Moreover, vitamin B_{12} seems to play an important role in myelination as its deficiency leads to demyelination and may cause severe retardation of myelination during prenatal development [54].

Different mechanisms have been proposed to account for the effects of vitamin B_{12} deficiency on general neural function, most especially in relation to

neurodevelopment. The most well-understood mechanisms are related to the metabolic reactions in which vitamin B_{12} is the exclusive cofactor for mammalian cells [55], and the importance of the coenzyme forms of vitamin B_{12}, methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl) to the methionine and methyl groups that are used for DNA synthesis, epigenetic and cellular division.

When vitamin B_{12} is in MeCbl form it is a cofactor for methionine synthetase, which promotes the methylation of homocysteine (HCY) to methionine required in cases of a reduced methionine status of dietary ingestion. The synthesised form of methionine is subsequently condensed into SAM, which is finally demethylated into S-adenosylhomocysteine (SAH) and is a methyl donor for the conversion of phosphatidylethanolamine to phosphatidylcholine [56]. The altered SAM:SAH ratio may be the result of B_{12} deficiency as SAH and HCY levels increase while SAM levels decrease. This decreased SAM:SAH ratio may impair the methylation that is necessary for the synthesis of proteins, lipids, and neurotransmitters [57, 58] and leads to inhibition of DNA synthesis and cell division, since folate is not being recycled [59]. These results show that without methionine, the myelin and neurotransmitters considered essential for neurodevelopment cannot be produced. Methionine

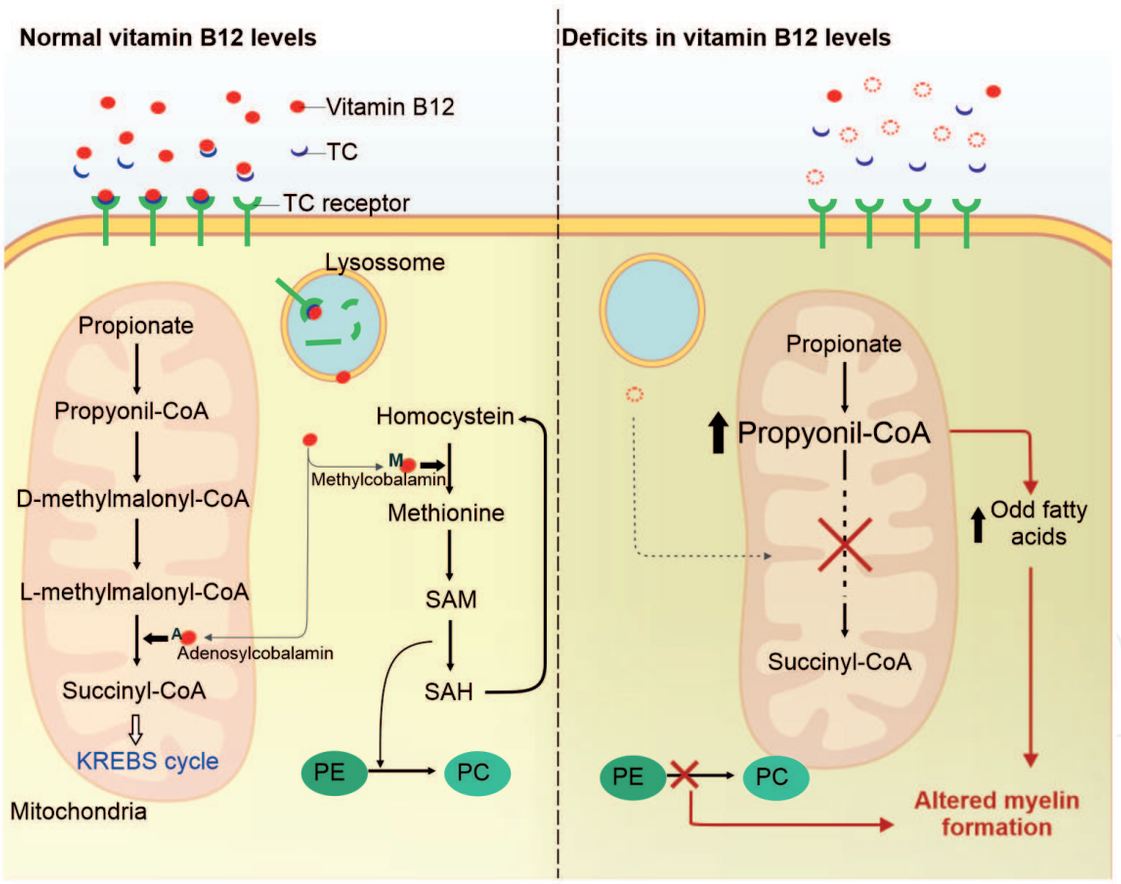

Figure 4.
Cellular processing of vitamin B in normal and deficiency conditions. Transcobalamin (TC) binds to vitamin B12 with high affinity and transports it by TC receptor-mediated endocytosis. The TC undergoes degradation into the lysosome, liberating the vitamin B12, that can be in two forms, methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl). When the vitamin B is in MeCbl form, it participates in HCY cascade as a cofactor, promoting the methylation of HCY to methionine that is subsequently condensed into SAM, which is finally demethylated into S-adenosylhomocysteine (SAH) and it a methyl donor for the conversion of phosphatidylethanolamine (PE) to phosphatidylcholine. The propionate (propionic acid) reacts with coenzyme A yielding propionyl CoA that is converted first to D-methylmalonyl-CoA and then to L-methylmalonyl-CoA and finally to Succinyl-CoA with the participation of AdoCbl form of vitamin B12 in this step. The deficiency of vitamin B12 leads to an inappropriate conversion of methylmalonyl CoA to succinyl CoA resulting in excessive production of the propionyl CoA, and consequently an increase of odd-chain fatty acid synthesis, and the inefficient conversion of PE to PC may impair propionyl CoA production by the mitochondria, resulting in an altered myelin formation.

is synthesised from homocysteine, increased levels of which are observed in many neurodegenerative diseases, and which are functionally linked to brain injury and cognitive impairment [60].

When vitamin B_{12} is found in adenosylcobalamin (AdoCbl) form, it is required as the cofactor of the enzyme L-methylmalonyl-CoA mutase (EC 5.4.99.2) in the mitochondria and promotes the conversion of methylmalonyl CoA to succinyl CoA. This pathway is important for the mitochondria to reuse propionyl CoA and the energy obtained through the Krebs cycle. In other instances, this pathway may be recruited in response to increased levels of SAM in which the elimination of HCY is necessary [61]. The inappropriate conversion of methylmalonyl CoA to succinyl CoA leads to excessive production of the precursor propionyl CoA, resulting in odd-chain fatty acid synthesis and subsequent incorporation of these abnormal fatty acids into the nerve sheaths, which leads to altered myelin formation, reduced quantities of ethanolamine, phospholipids, and sphingomyelin (**Figure 4**) [56]. In addition, the inefficient conversion of phosphatidylethanolamine to phosphatidylcholine may impair propionyl CoA production by the mitochondria and the myelination, or lead to demyelination [62]. Myelination, together with synaptic refinement and the physiological maturation of inhibitory neural networks, are key processes of brain development that seem to coincide with the appearance of PNNs. In fact, alterations in these processes are well described in disorders such as SZ and ASD [63, 64].

3. Vitamin D and neurodevelopment

Vitamin D is known as the "neglected neurosteroid", a term proposed by McGrath and colleagues in 2001 [65], and exerts a great variety of effects during brain development.

In 2005, a study conducted the first description of the distribution of 1,25-dihydroxyvitamin D3 receptors (VDR) and 1α-hydroxylase (1α-OHase), the enzyme responsible for the formation of the active vitamin D, in the human brain. They are found both in neurons and glial cells and show a region- and layer-specific pattern of expression in the brain. VDR receptors are absent in the macrocellular cells within the nucleus basalis of Meynert (NBM) and Purkinje cells in the cerebellum, while both VDRs and 1α-OHase have been identified in the substantia nigra and the hypothalamus [66]. VDRs can also be found in the developing brain during the critical period of cell proliferation, in the temporal lobe, cingulate, thalamus, cerebellum, amygdala and hippocampus [67].

Similarly to vitamin B_{12} deficiency, vitamin D deficiency during pregnancy appears to have serious consequences for foetal health. In the literature, abortions within the first trimester of gestation have been reported in association with the mothers' lack of sufficient vitamin D concentration [68–70]. Moreover, as foetus are dependent on access to the mother's supply of vitamin D, in conjunction with the large distribution of vitamin D receptors in the brain, there is a high probability that the vitamin D status of pregnant mothers may affect child neurocognitive development [67]. Among other sequelae, researchers have found cognitive and neural deficits in foetus and young infants due to maternal vitamin D deficiency during pregnancy, such as suboptimal neurocognitive development [71] and delays in the development of gross and fine motor function, problem-solving and communication [72]. However, a recent study analysed high-dosage vitamin D supplementation in the third trimester of pregnancy and its effects on child brain development and failed to report any improvements in the neurobiological mechanisms underlying developmental behaviour, such as motor milestones and cognitive and language development [73]. Few studies of the direct effects of vitamin D on

brain development have been conducted to date in humans and many of them are inconclusive, probably because of differences in the timing of vitamin D exposure, the types of assessment used, and the age at which the assessment occurred.

Studies in rats have identified similar critical periods to vitamin B_{12} during neurodevelopment in which maternal vitamin D deficiency can result in neurodevelopmental alterations, such as abnormal cell proliferation and decreased expression of neuronal structure genes in the brain of mice offspring [74]. VDRs are temporally regulated in the rat brain during development, with the first VDR expression occurring in the mesencephalon on day 12 [75]. VDR expression continues across different areas throughout the course of development [75, 76] and is directly correlated to the onset of natural cell elimination [77]. Because of its key role in regulating developmental processes throughout the brain, altered levels of vitamin D or VDR dysfunction can result in long-lasting behavioural disruption in animal models [67]. Consistent with its role in PNN formation, altered vitamin D levels and VDR signalling during development have been associated with neuropsychiatric disorders such as SZ [78] and autism [79, 80].

The mechanisms by which vitamin D influences brain development are diverse. There is evidence that vitamin D modulates neurotrophic factors such as NGF and glial cell line-derived neurotrophic factor (GDNF), suggesting that it may have a neuroprotective function [81, 82]. Other neuroprotective functions of vitamin D include the reduction of the neurotoxicity of glutamate and reactive oxygen species (ROS), the upregulation of antioxidant molecules [83, 84], and the suppression of macrophage activity, leading to decreased neuroinflammation activity [85]. When used as an immune suppressant, vitamin D has been reported to suppress the concentration of proinflammatory cytokines in the brain [86, 87], to reduce blood–brain barrier disruption and macrophage/microglia activation in induced autoimmune encephalomyelitis [88], and to attenuate proinflammatory processes and up-regulate anti-inflammatory processes such as the M2 microglia phenotype, in a mouse model of Parkinson's disease [89].

Vitamin D is known to reduce calcium levels in the brain, preventing cell death via excitotoxicity, and to downregulate or modulate L-type voltage-gated calcium channels (L-VGCCs) [90] (**Figure 5**). L-VGCCs are expressed in great quantity in the developing brain and have a critical role in synaptic plasticity and in regulating basal and burst firing activity in dopaminergic neurons within the ventral tegmental area [91]. Interestingly, disruption of L-VGCC function in hippocampal PV^+ interneurons during development leads to significant morphological changes, such as reduced cell number and a decrease in dendritic arbour complexity [92, 93].

The association between vitamin D and L-VGCC function in the dopaminergic system and PV^+ interneurons strengthen the hypothesis that vitamin D (dys) regulation plays a role in the onset of SZ [94]. Both epidemiological research and rodent models have shown a correlation between vitamin D deficiency and SZ. Alterations in the dopaminergic system have been frequently reported in response to vitamin D deficiency, consistent with increased VDR expression in brain areas primarily innervated by dopaminergic projections [95].

Associations have also been made between vitamin D receptors, calcium channels, PV^+ interneurons and PNNs. L-VGCCs regulate PV^+ expression and interneuron development [92] which are significantly disrupted in SZ. Curiously, the appearance of PNNs coincides with the synaptic refinement, myelination and maturation of inhibitory networks, and there is therefore strong evidence that PNNs have a pivotal role in the pathogenesis of SZ [63].

Following this same reasoning, vitamin D deficiency is considered a potential candidate for the development of several key alterations in ASD pathophysiology, most of them present mid to early development. John Cannell has been the main

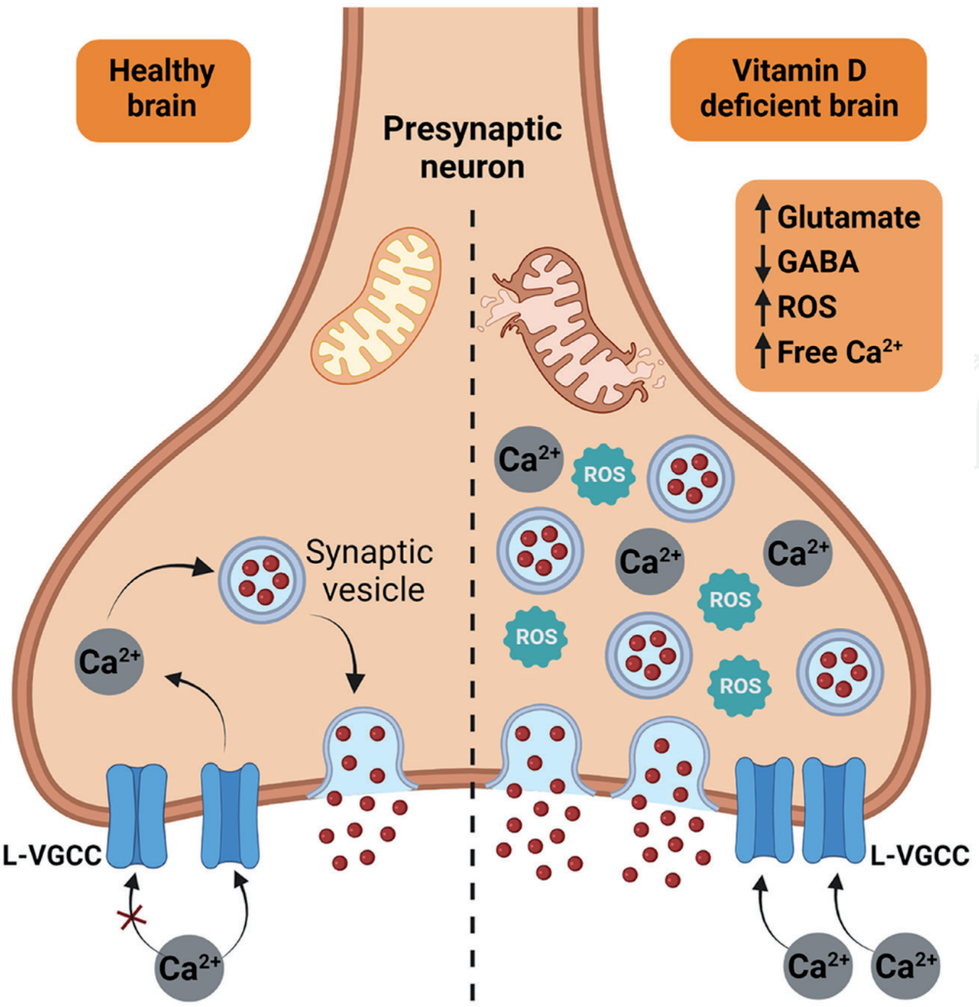

Figure 5.
Effects of vitamin D in presynaptic transmission Vitamin D plays an important role in several neurodevelopmental processes, such as neurotransmission and calcium signalling, preventing cell damage, especially mitochondrial dysfunction. As mentioned above, vitamin D acts by regulating L-VGCC in order that, for example, prevent the accumulation of intracellular calcium and the concomitant activation of pathways that can lead to cell death. With vitamin D deficiency, L-VGCC becomes functionality altered, leading to increased intracellular calcium, mitochondrial dysfunction, and the consequent generation of free radicals such as reactive oxygen species (ROS) and inhibitory/excitatory synaptic imbalance, illustrated by the increase in glutamate release and GABA neurotransmitter decrease, resulting in excitotoxicity and neuroinflammation (modified from [91]). Created with BioRender.

proponent of this hypothesis [96] and since its inception a number of studies have contributed to this line of research. The pathophysiology of ASD is multifactorial with solid evidence for both a genetic background and an environmental component. Some epidemiological findings have raised the additional hypothesis of specific genes which are environmentally responsive to the ASD, as epidemiological observations have pointed to changes in genotype expression. Cannell and others have cited neurosteroid pathway genes as good examples of environmentally responsive genes, since alterations in neurosteroid concentration may affect the genetic expression of the neural proteins regulated by steroids.

4. The link between vitamins B_{12} and D, PNNs and neurodevelopmental disorders: a mechanistic hypothesis

As already mentioned before, PNNs appear during postnatal development and surround cortical inhibitory GABA neurons expressing PV^+, which control

the output of mainly cortical and hippocampal neurons and are necessary for fast rhythmic neuronal synchrony during information processing in cognitive tasks [97, 98]. PNNs enveloping PV^+-inhibitory interneurons are known to be vital for cognition [99]. These cortical PV^+-inhibitory interneurons express specific NMDA receptor (NMDAR) subunits such as NR2A, and are targets of the NMDA receptor antagonist MK-801. MK-801 is the main component for studying an important hypothesis for our understanding of the aetiology of SZ, the cortical hypofunction of NMDA receptors (i.e. the glutamatergic hypothesis) [100]. This hypothesis is compatible with the concurrent descriptions provided by the neurodevelopmental hypothesis, with respect to the disruptions to the central nervous system over the course of development [101].

An animal model using MK-801 treatment revealed a critical postnatal period, specifically from 7 to 14 days after birth (P 7–14), resulting in SZ-like behaviour during adulthood, with a significant reduction in PV^+-expressing cells in the PFC but not in the hippocampus, for mice treated with MK-801 from P 7–14 compared to matched controls [102]. In addition, mice in the treated group showed changes in performance on cognitive, social and behavioural tasks, while electrophysiological recordings from brain slices within the PFC showed significantly reduced frequency of up-states in MK-801-treated mice, but increased gamma activity in MK-801-treated mice compared to saline-treated mice [102]. Recent memory reactivation has been shown to occur in the presence of slow oscillatory up-states, contributing to memory consolidation [103]. This electrophysiological profile is altered in humans with SZ, with increased delta oscillations and irregular gamma rhythm [104, 105]. Moreover, PNN removal from visual cortex during a critical postnatal period alters the balance between excitatory and inhibitory PV^+ spiking activity, inducing greater potentiation of gamma activity and restoring the neural network to an immature or juvenile ECM state, suggesting that the maturation of GABAergic fast spiking and PV^+-inhibitory neurons suppresses the spontaneous activity of excitatory neurons [106].

Together with these electrophysiological findings, the metabolic requirements of PNN function have also been explored in fast-spiking cells, as well as their intrinsic vulnerability. In fact, PNNs appear to promote interneuron maturation and network stability and may also protect neurons against iron sequestration and oxidative stress [107, 108]. Oxidative stress has been observed in the PFC of SZ patients in conjunction with decreased levels of glutathione (GSH), an endogenous antioxidant and redox regulator [109]. This outcome can in turn be aggravated by the overexpression of truncated DISC1 that is associated with SZ in humans, causing mitochondrial dysfunction with decreased mitochondrial NADH dehydrogenase activity, diminished cellular ATP contents, and overactivity of mitochondrial $Ca2^+$ mechanisms [110].

As mentioned previously with regards to the role of GSH, there are two metabolically active forms of vitamin B_{12}, AdoCbl, and MeCbl: essential as a cofactor for the reaction necessary folate-dependent methylation of HCY by methionine synthase (MS) in the cytoplasm. As MS levels determine the ratio of methyl donor SAM to the endogenous methylation inhibitor SAH, the MeCbl reaction can influence SAM-dependent methylation reactions mainly through methylation of DNA and histones [111], an effect previously described with in relation to BDNF and epigenetic control. Zhang et al. 2016 analysed the postmortem human frontal cortex of autistic and schizophrenic individuals and 80-year-old individuals and found that the MeCbl form of vitamin B_{12} decreases with age, as well as to age of onset of ASD and SZ, as compared to age-matched controls. Additionally, they also observed an abnormally lower total cobalamin Cbl and MeCbl concentration in ASD and SZ subjects, leading the authors to propose a "Redox/Methylation Hypothesis of Autism" in light of the impaired GSH-dependent synthesis observed in the brains of autistic individuals [111]. In the same study, the authors further found that certain

brain regions, as cerebellum and temporal cortex, in ASD, showed synthesis of reduced and oxidised glutathione (GSH/GSSG) (stable redox status), while other regions maintained SAM/SAH production (stable methylation status) [111], suggesting regional differences for metabolic disorders.

Concomitantly, several neurobiological changes found in ASD subjects or ASD animal models are consistent with an account of vitamin D deficiency. The main neurobiological processes mediated by vitamin D in neurodevelopment include neural cell proliferation [74], GABA, glutamate and serotonin neurotransmission [92], calcium signalling, mitochondrial regulation, oxidative stress and neuroinflammation (for review see [112]). Most of these processes are altered in ASD, resulting in the reduction of brain volume and changes in the number of glial and neuron cells, excitatory/inhibitory imbalance, disrupted calcium signalling, increased oxidative stress, the overactivation of microglia, and immune system dysregulation [113].

One of the best-studied hypotheses about the pathophysiology of ASD is the occurrence of changes in brain connectivity during development, which posits a reduced number of connections between distal brain regions and an increase in connections between proximal brain regions [114]. However, this hypothesis shows some inconsistencies in the light of several findings, such as reports of hyperconnectivity over long axonal fibres in autism or even mixed patterns of hypo- and hyper-connectivity [115–118].

Another hypothesis regarding the pathophysiology of ASD is one of excitatory-inhibitory imbalance, caused both by the probable hyperactivity of excitatory cells and a reduction in the number, activity or even delay of inhibitory interneurons in the maturation process [119–121].

PV^+-expressing interneurons are the main regulators of inhibitory/excitatory balance and orchestrate the coordinative function of brain microcircuitry via their fast-spiking inhibitory inputs onto pyramidal neurons [97, 122]. As such, the healthy maturation of PV^+-interneurons is crucial for the establishment of optimal neural and behavioural development. Disruption to PV^+ expression in PFC caused by early-life adversity leads to altered social interactions [123] and anxiety-related behaviour in rodents [124, 125]. Both forms of altered behaviour can be found in patients diagnosed with ASD [126–128].

As mentioned previously, the maturation of PV^+ interneurons are mostly regulated by PNNs [129, 130]. Despite the protective actions of the PNN-PV^+, the interneurons are very susceptible to oxidative stress [131, 132], and as such vitamin D deficiency represents a threat to the integrity of PNN function and consequently to the development of the GABAergic system.

Lastly, it is important to highlight the dysregulation of the immune system that is observed in ASD. ASD patients suffer from chronic systemic inflammation with a disbalance in cytokine expression, leading to increased production of proinflammatory cytokines. Vitamin D has been shown to contain immunomodulatory properties and may be an alternative treatment for slowing or minimising behavioural alterations in ASD (for review see [133]). Chronic systemic inflammation in early life may disrupt PV^+ interneuron maturation and consequently lead to changes in PNNs. Taken together, these findings reveal the important role of vitamin D in maintaining the integrity of PNNs and the efficiency of synaptic transmission as a whole.

5. Conclusion

The ECM is one component of the tetrapartite synapse, together with the network of glial cells. The PNN is a dense ECM structure that enwraps inhibitory fast-spiking parvalbumin (PV^+) interneurons, serving both as a protective barrier

and to regulate synaptic plasticity. The destruction of those PNNs during the critical postnatal period of brain development alters the balance between excitatory and inhibitory PV^+ spiking activity, inducing a greater potentiation of gamma-band activity, and reverting the firing pattern of the neural network to a so-called immature or juvenile ECM state. Studies have shown that a vitamin D deficiency in early development may lead to a reduction in the PNN integrity and synaptic plasticity through modulation of MMPs, contributing to increased risk of the onset of neuropsychiatric disorders, as SZ, ASD, and ADHD. Also, in preclinical studies, dose-dependent vitamin B_{12} supplements were sufficient to maintain the levels of DHA and BDNF in the hippocampus and cortex in neonates, and BDNF levels in the hippocampus at 3 months of age, considered a sensitive window or critical period during neurodevelopment. In addition, the ingestion and metabolism of vitamin B_{12} methylcobalamin (MeCbl) variants can influence S- adenosyl methionine and SAM-dependent methylation reactions, mainly through the methylation of DNA and histones, and the MeCbl deficiency can result in impairment of GSH-dependent synthesis, inducing oxidative stress in the PFC of schizophrenic patients, or with autism diagnosis. Thus, adequate levels of vitamin B_{12} and D appear to contribute to maintaining the integrity of PNNs, consequently lead to PV^+ interneuron maturation.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Marcela Bermudez Echeverry[1,2*], Silvia Honda Takada[1], Bruna Petrucelli Arruda[1], Debora Sterzeck Cardoso[1], Pamela Pinheiro Martins[1], Juliane Midori Ikebara[1], Aline V. Sousa-Santos[1] and Victor R.C. Torres da Silva[1]

1 Universidade Federal do ABC (UFABC), Centro da Matemática, Computação e Cognição, São Bernardo do Campo, SP, Brazil

2 Neuroscience Laboratory, School of Medicine, University of Santander (UDES), Bucaramanga, Santander, Colombia

*Address all correspondence to: marcela.echeverry@ufabc.edu.br

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