Introduction

Prolactin (PRL) is a pleiotropic hormone responsible for many biological functions that go far beyond its roles in reproduction and lactogenesis, like the modulation of the immune system, growth and metabolism, osmoregulation and regulation of brain function. These are well-documented roles of PRL in mammals, as well as in other vertebrates (Bernard et al., 2019; Dobolyi et al., 2020). The effects of PRL on the mammalian brain depend on factors such as age, gender, and reproductive status (Lajud et al., 2013; Salais-López et al., 2018; Phillips et al., 2019). The functions of PRL in the brain are particularly relevant during pregnancy and lactation which are characterized by high levels of PRL (Phillips et al., 2020). PRL induces neurogenesis during pregnancy in mice (Shingo et al., 2003; Ueno and Grattan, 2010; Wang et al., 2013) and parental behaviors, which have been conserved along evolution, from fish to mammals (Dobolyi et al., 2020), and modulates other brain functions (Leem et al., 2019; Vermani et al., 2020).

The main goal of this review is to highlight the most recent findings on the effects of PRL in the function of the mammalian brain. A brief overview of the main signaling cascades elicited by PRL in the brain and the distribution of extrapituitary sources of PRL in the central nervous system is initially provided followed by a thorough review on the role of PRL in the induction of neurogenesis, neuroprotection and neuroplasticity. The therapeutic potential of this neuropeptide in brain disorders is also discussed.

Search Strategy and Selection Criteria

Research articles published between January 2016 and May 2021 were retrieved from the PubMed database using the following terms: prolactin, brain, parental behavior, prolactin receptor signaling, neurogenesis, neuroprotection, neuroplasticity, and neurological disorder. Although bibliographic revision focused on the last 5 years, earlier mandatory references in the field were also considered.

Prolactin Structure and Prolactin Variants

PRL is a polypeptide hormone mainly produced in the lactotrophs of the anterior pituitary gland (Bernard et al., 2019). The mature PRL protein of pituitary origin is composed of 197–199 amino acids in mammals (Grattan, 2015). PRL appeared early in evolution and remain relatively conserved (Dobolyi et al., 2020), presenting high homology levels in mammals (Table 1). In vertebrates, PRL is encoded by a single gene composed of 5 exons and 4 introns (Bernard et al., 2015). However, despite sharing some common structural and regulatory features, the PRL gene is distinct between species. For instance, the human PRL gene contains an additional noncoding exon. This superdistal promoter region, firstly identified in the human decidua, is responsible for the regulation of its transcription at extrapituitary sites (Hiraoka et al., 1991).

PRL is part of a family of polypeptide hormones that have similar structural and biological characteristics, which includes the growth hormone and placental lactogen. Human PRL is a 23 kDa protein, structurally composed by four antiparallel α-helices (Molina-Salinas et al., 2021). Moreover, several other PRL isoforms, resulting from proteolytic cleavage, alternative splicing and post-translational modifications like glycosylation, dimerization and association with other circulating proteins, have also been described (Bernard et al., 2015).
It is hypothesized that different PRL isoforms have distinct functions and biological activity, contributing in part to the plethora of PRL functions. For instance, in humans, macroprolactin (> 100 kDa) has reduced biological activity (Fahie-Wilson and Smith, 2013). On the other hand, vasohibin (a 16 kDa PRL isoform) is a potent anti-angiogenic factor, while 23 kDa PRL seems to have angiogenic properties (Struman et al., 1999).

Prolactin Action Is Mediated by Its Receptors

PRL receptors (PRLR) belong to the type I cytokine receptor family and are composed by three domains: extracellular, transmembrane, and intracellular (Bugge et al., 2016). Similarly to other members of this family, the action of PRL is initiated when it binds to two PRLR units, forming a biological activated heterotrimeric complex (Bridges and Grattan, 2019). It is important to notice that other hormones, like growth hormone and placental lactogen, also bind PRLR (Bernard et al., 2015), and that in pregnancy, part of PRL signaling may be induced by placental lactogen (Phillips et al., 2020). Alternative splicing of the PRLR gene results in several PRLR isoforms with distinct intracellular domain lengths that vary between species. While rats have three distinct PRLR isoforms, long, short and intermediate (Bernard et al., 2019; Molina-Salinas et al., 2021), several other additional PRLR isoforms, including two PRLR-short forms and a secreted soluble form, are present in humans (Trott et al., 2003). However, the differences between the intracellular domain of the PRLR do not have an impact in the elementary functions of the PRL, as observed in 84 mammalian species (Paré et al., 2021).

PRL binding to the long form of PRLR can elicit distinct signaling cascades mediated by tyrosine kinase activation (Figure 1), including Janus protein kinase 2 (JAK2) autophosphorylation and JAK2-mediated tyrosine phosphorylation of the receptors (Bernard et al., 2015). The JAK2 activation is the best characterized and one of the fastest mechanisms of action mediated by the PRLR-long form (Campbell et al., 1994). Binding of PRL to the PRLR-long form induces JAK2-mediated phosphorylation of signal transducer and activator of transcription (STAT) proteins that translocate to the nucleus. In the nucleus, the STAT proteins bind to gene promoters that contain the y-interferon activated sequence DNA-binding motif and regulate the transcription of PRL target genes (Gilmour et al., 1995), constituting the canonical JAK/STAT pathway. In turn, JAK/STAT signaling is inhibited by proteins of the suppressor of cytokine signaling (SOCS) family (particularly SOCS1 and SOCS3), by a negative-feedback loop promoted by the activation of JAK/STAT pathway. SOCS proteins contain a Src homology 2 domain and a kinase-inhibitory region (KIR) that inhibits the kinase activity of JAKs interfering with JAK/STAT signaling (Durham et al., 2019).

The mitogen-activated protein kinase (MAPK)/extracellular signal regulated kinase (ERK) is another signaling pathway activated by PRL (Figure 1). Evidences from studies in human and mouse mammary epithelial cell lines, revealed that the activation of this pathway involves upstream intermediaries like SHC, growth factor receptor-bound protein 2, SOS, Ras and Raf (Das and Vonderhaar, 1996), that lead to the phosphorylation of MAPK and ultimately regulate cell proliferation (Piccoletti et al., 1994; Acosta et al., 2003). Another signaling cascade associated with the PRLR-long form signaling described in human breast cancer cell lines, is phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway (Figure 1). Briefly, PRL binding to PRLR activates Src, mediating the activation of PI3K and AKT that in turn modules cell proliferation (Acosta et al., 2003). The exact mechanism of action of PRLR-short forms is unknown since the activation of JAK/STAT pathway is prevented. However, the PRLR-short isoform is able to activate the MAPK and PI3K/AKT pathways (Bridges and Grattan, 2019; Wen et al., 2020; Molina-Salinas et al., 2021). PRLR-short signaling was also associated with the inhibition of FOXO3 expression in the mice ovary, resulting in ovarian developmental defects (Halperin et al., 2008). On the other hand, the blockage of PRLR-short form with an antagonist in human uterine cancer cells lines was associated with a reduction in cell proliferation, possibly mediated by the reduction of PI3K/AKT and augmented FOXO3 nuclear translocation (Wen et al., 2020). Conversely, the activation of the PRLR-short form in pancreatic ductal adenocarcinoma mice and in human models reduced cell proliferation. In this case the reduced cell proliferation was associated with decreased expression of genes involved in the pentose phosphatase pathway, through the activation of the Hippo signaling pathway (Nie et al., 2021). Even though the available information regarding PRLR-short form signaling is conflicting, and that PRLR-short, like the PRLR-long signaling cascade, may be tissue and physiological state dependent, all data seem to reinforce that the PRLR-short form is involved in the modulation of relevant biological actions.

Despite sharing the same signaling cascade, the target genes regulated by PRLR activation are cell type dependent. For instance, while in many cells PRL was associated with antiapoptotic and proliferative effects, in rat osteocytes, PRL elicited apoptotic and antiproliferative effects instead (de Dios et al., 2019). In this case, the PRL-induced apoptotic and antiproliferative effects were mediated by the JAK2/STAT5 pathway and inhibition of both ERK and Akt phosphorylation (de Dios et al., 2019). It is important to recognize that most studies dedicated to the investigation of type I cytokine receptor signaling cascades were performed in cancer cells, and that information regarding the biological actions mediated by this receptor family in healthy cells is still lacking.

Prolactin Signaling in the Brain

In mammals, PRLR are expressed in brain regions (Figure 2), like the amygdala, the preoptic area, the thalamus, the hypothalamus, the epithalamus and the brainstem (Kokay et al., 2018; Voigt and Bennett, 2018; Szczesna et al., 2020). It is important to notice that most studies that investigated the expression of PRLR in the brain used probes that do not discriminate between short and long forms of PRLR. PRLR are also present in the subventricular zone (SVZ) and in the hippocampus in mice (Mak and Weiss, 2010; Anagnostou and Morales, 2019), as well as in the circumventricular organs, like the median eminence, the subfornical organ and the choroid plexus (CP) in rodents (Kameshi et al., 2018; Kobayashi et al., 2018; Voigt and Bennett, 2018). In fact, the CP is the region of the brain with the highest expression of PRLR-long form (Kokay et al., 2018). This PRLR form is located in the cytoplasm and both the apical and the basal membrane of rat CP epithelial cells (Costa-Brito et al., 2021).
Phosphorylation of STATS, mediated by activated PRLR-long form, is considered to be the main PRL signaling pathway in the mouse brain (Yip et al., 2012), especially during pregnancy (Salais-López et al., 2017; Gustafson et al., 2020). However, neuronal ablation of STATS in mice revealed that STATS phosphorylation is not mandatory for the expression of nursing behaviors. In fact, the induction of faster responses in the maternal behavior does not exclude that PRL-mediated effects in this brain region do not always involve the activation of transcription factors but rather other unidentified mechanisms of action (Buonfiglio et al., 2015).

Increasing evidences support the hypothesis that PRL elicits fast and transient calcium channels/transporters responses in specific subsets of hippocampal and hypothalamic neurons in mice (Leem et al., 2019; Georgescu et al., 2020). Recently, PRL-mediated action was associated with the modulation of membrane excitability in the neurons of the subfornical organ (Kamesh et al., 2018) and the arcuate nucleus (Blum et al., 2019) in rodents, with a particular relevant role of the transient receptor potential cation channel TrpC5 in the later. Other PRL-induced responses in the brain include the activation of ERK1/2 MAPK pathway in the hypothalamus and in hippocampal progenitor cells in rats (Blume et al., 2009; Wagner et al., 2009), or AKT phosphorylation in the hippocampus of male mice (Anagnostou et al., 2021).

The expression of the PRLR-short form has been reported in hypothalamic areas, the amygdala, the thalamus and the CP of female rats (Pi et al., 2002; Bakowska and Morrell, 2003), and in the SVZ and the hippocampus of male mice (Mak and Weiss, 2010). Overall, PRLR-long and PRLR-short expression seem to overlap in several brain regions, but the expression of the PRLR-long form is usually higher than that of the PRLR-short (Bakowska and Morrell, 2003). Furthermore, the increase of both PRLR forms in the rat brain during lactation (Pi and Grattan, 1999) and the observation of PRL actions mediated by PRLR-short form in the mice nervous system (Belugin et al., 2013) have also been reported. Together, these evidences suggest that the PRLR-short isoform may be involved in PRL-mediated brain functions in rodents. In addition, PRLR isoforms can interact and regulate the action of each other in both mice and humans (Belugin et al., 2013; Kang et al., 2014). For instance, in mice, the transient action of PRL mediated by the PRLR-short form in sensory neurons of the trigeminal ganglia is negatively regulated by the presence of PRLR-long form in these neurons (Belugin et al., 2013). However, most studies involving the investigation of PRLR activation in the brain do not discriminate between isoforms of these receptors, PRL signaling in the brain may not always be mediated by the activation of the canonical PRLR-long form pathways.

**Sources of Prolactin in the Brain**

Based on its size, PRL should not cross the blood-brain barrier (BBB). However, PRL is present in the cerebrospinal fluid (CSF), mimicking the maternal circulation, suggesting that PRL transport into the brain must occur (Login and MacLeod, 1977). For instance, in mice, transport of PRL into the brain is increased during lactation (Brown et al., 2016), a physiological state characterized by high levels of serum PRL. Based on the high expression of PRLR in the CP, and on the evidence depicted from studies in primates, a saturable receptor-mediated mechanism of transport of PRL across the CP has been considered the likely route of entrance of PRL into the CNS (Walsh et al., 1987). The CP, located within the brain ventricles, is responsible for the production of CSF and several peptides, as well as for controlling the passage of molecules into the brain (Talhada et al., 2019), that lack the PRLR transcripts in the CP, identical to those expressed in the pituitary, the PRL detected in the CP has a much higher molecular weight than pituitary PRL or PRL secreted to the conditioned media of pituitary cultures. This high molecular weight immunoreactive-PRL was detected in CP conditioned media of rat CSF and rat CP cell conditioned media and in the rat CSF (Costa-Brito et al., 2021). The existence of high molecular weight PRL isoforms similar to those found in the CP was previously described in other cells like human monocytes (Lépincón et al., 2013). However, the biological activity and relevance of this high molecular weight PRL remains controversial. The recognition of non-pituitary sources of PRL in the brain is not consensual either, with some investigators defending that the reported expression of PRL mRNA based in high sensitivity techniques like RT-PCR may be the result of sample contamination with other cells that express PRL (Bridges and Grattan, 2019). Overall, considering that the expression of PRL in brain regions other than the pituitary is very low, it is unlikely that PRL produced in extratypic tissues may have some impact in the circulating levels of PRL. Nevertheless, it is possible that brain PRL from extratypic sources could be produced in specific physiological circumstances, which require PRL autocrine and paracrine modulation of brain functions and account for the levels of PRL in the CSF.

**Prolactin Actions in the Brain**

The best documented actions of PRL in the mammalian brain, are associated with mechanisms that prepare the brain to motherhood/ fatherhood (Larsen and Grattan, 2010; Mak and Weiss, 2010; Brown et al., 2017; Stagkourakis et al., 2020). In female mice, the action of PRL in specific areas of the brain, including the medial preoptic area region of the hypothalamus and SVZ, is essential for the development of maternal behaviors like retrieving and grooming over the pups (Larsen and Grattan, 2010; Brown et al., 2017). In turn, male mice, a biparental species, and rat males, a uniparental species, display different paternal behaviors, which may be explained by distinct release profiles of PRL between species (Stagkourakis et al., 2020). Yet, the administration of PRL can elicit fatherhood behaviors even in males of non-paternal species like rats (Stagkourakis et al., 2020). Furthermore, PRLR expression is relatively similar in the brain of male and female mice (Kokay et al., 2018), reinforcing the theory that PRL may also play a relevant role in male brain functions of biparental species. On the other hand, in sheep, the increased expression of PRLR in the arcuate nucleus, contrast with the reduction of PRL levels in the median eminence and in the adenohypophysis, during gestation. This may suggest that, during sheep gestation, PRL may have a predominant role in the regulation of appetite (Szczesna et al., 2020).

In rodents, the fine-tuned balance of PRL levels during pregnancy and postpartum is fundamental to the development of healthy maternal behavior of the progenitors and of the offspring in their adult life. Abnormal levels of PRL during prenatal and early-life in mice, have a negative impact on the development of social behaviors of the male offspring (Barad et al., 2020; Mitani et al., 2018). More recently, the genes modulated by PRL in the hippocampus of female rats were examined by RNAseq. A
The action of prolactin is initiated by its binding to a prolactin receptor (PRLR) homodimer, forming a heterotrimERIC complex. PRL binding to the long form of PRLR triggers distinct signaling cascades that ultimately culminate in the regulation of gene transcription. The main activation pathway of PRL is the JAK/STAT pathway, but prolactin is also able to induce the PI3K/AKT pathway. The MAPK/ERK is another signaling pathway triggered by prolactin binding to both PRLR isoforms. The activation of this pathway involves the SHC, Grb2, SOS, Ras and Raf cascades. It is also believed that the activation of the PRLR-short form inhibits the expression of FOXO3 and Galt, involved in the metabolism of galactose to glucose, possibly leading to developmental defects. However, the exact mechanism of action of this pathway remains unidentified. AKT: Protein kinase B; ERK: extracellular signal regulated kinase; FOXO3: forkhead transcription factor 3; Galt: galactose-1-phosphate uridylytransferase; Grb2: growth factor receptor-b distract protein 2; JAK2: Janus kinase 2; MAPK: mitogen-activated protein kinase; MAPKK: MAPK kinase; PI3K: phosphatidylinositol 3-kinase; Ras: Rat sarcoma; SHC: SHC-transforming protein 1; SOS: son of sevenless; Src: proto-oncogene tyrosine-protein kinase Src; STAT5: signal transducer and activator of transcription 5.

In mice, PRL modulates neurogenesis in the SVZ, where progenitor cells differentiate into interneurons that migrate to the olfactory bulb (Shingo et al., 2003), and in the subgranular zone of the hippocampal dentate gyrus (Figure 3). These are the two brain neurogenic niches where progenitor cells continue to differentiate in new neurons and glial cells in adult life (Mak and Weiss, 2010; Walker et al., 2012). In the SVZ, PRL plays an important role in the formation of the olfactory bulb by increasing the proliferation of neural stem cells during the early stage of pregnancy and early postpartum period in mice that further differentiate in olfactory neurons (Shingo et al., 2003). PRL also raises SVZ neurogenesis in male mice that interact with their pups (Mak and Weiss, 2010). In mice, PRL-induced adult SVZ neurogenesis is mediated by the ERK5 signaling pathway (Wang et al., 2013) and is fundamental for the development of healthy maternal behaviors (Larsen and Grattan, 2010), offspring recognition (Shingo et al., 2003; Mak and Weiss, 2010) and choice of mates by females (Mak et al., 2007). Additionally, exposure of females to male pheromones seems to be one of the factors that regulate PRL-induced neurogenesis. Besides increasing PRL levels in female mice, pheromone exposure mediates the generation of new neurons in the SVZ in a PRL dependent manner (Mak et al., 2007; Larsen et al., 2008). Although olfactory bulb neurogenesis seemed to be fundamental for the development of maternal behavior and lamb recognition in sheep (Corona et al., 2018), no direct observation between PRL and olfactory bulb neurogenesis has been reported in this species so far.

Considering that the CP is near the SVZ, the possible contribution of the CP prolactin (PRL) production to neurogenesis should also be considered. The CP epithelia is a remarkable source of peptides that modulate brain function and neurogenesis (Marques et al., 2017). In that regard, in a recent RNAseq study conducted to examine transcriptomic differences induced by PRL, insulin-like growth factor 2 (Igf2) has emerged as a gene highly responsive to PRL in the adult mice CP. The upregulation of the Igf2 gene during lactation, suggest that this particular growth factor is possibly involved in the postpartum increase of SVZ neurogenesis (Phillips et al., 2019). In fact, in the pioneer work of Shingo and colleagues, the authors suggested that due to the high expression of PRLR in the CP, that this structure could indirectly mediate PRL action in SVZ neurogenesis (Shingo et al., 2000). In local production of PRL in the rat CP (Costa-Brito et al., 2011), may explain the relevant role of the CP in PRL-induced neurogenesis in the SVZ.

The role of PRL in the neurogenesis in the dentate gyrus hippocampal progenitor cells is not consensual. Some studies describe that PRL enhances the proliferation of the dentate gyrus cells in both female and male mice (Mak and Weiss, 2010; Walker et al., 2012) and prevents the reduction of adult hippocampal neurogenesis promoted by chronic stress in male mice (Toner et al., 2009). Furthermore, PRL null mice display impaired learning and memory processes, and PRL infusion was able to restore the learning deficits observed in these mice. However, despite PRL-deficient mice presented lower generation of hippocampal precursor cells in vitro, the same was not observed in vivo, suggesting that in the absence of PRL, hippocampal neurogenesis may be sustained by other factors (Walker et al., 2012). Other studies report that PRL has either no effect on rodents and human neurogenesis (Shingo et al., 2003; Wagner et al., 2009;...
Prolactin (PRL) has been associated with modulatory actions in the two major neurogenic niches in adult brain, the subventricular zone (SVZ) and subgranular zone (SGZ) of the dentate gyrus (DG). In the SVZ, progenitor cells differentiate into interneurons that migrate throughout the rostral migratory stream (RMS) to the olfactory bulb. During the early stage of gestation and lactation, PRL increases the number of proliferating cells in the SVZ and raises the amount of new interneurons in the olfactory bulb. The role of PRL in hippocampal progenitor cells neurogenesis is not as consensual. Some evidences support that PRL favors DG cells proliferation, while others suggest that PRL has neither effect or that negatively impacts hippocampal neurogenesis. In addition, recent evidence suggested that the choroid plexus may also be an alternative source of PRL in the rat brain. However, further studies are necessary to understand if choroid plexus derived PRL has any effects in SVZ neurogenesis. LV: Lateral ventricle.

Michael Smeeth, et al., 2021, or that in fact it has a negative impact on hippocampal neurogenesis in rats (Lajud et al., 2013).

More recently, a study conducted in an in vitro model of human hippocampal stem cells revealed that PRL treatment was able to increase neuronal differentiation. However, this increase was only transient and continued PRL exposure actually decreased the number of proliferating cells, suggesting that fluctuations of PRL levels in different reproductive stages may induce short-term alterations or not contribute at all to adult hippocampus neurogenesis (Smeeth et al., 2021). Interestingly, in an earlier study conducted with a distinct human neural stem cell in vitro model, distinct PRL levels were associated with different neuroblast and glial progenitor proliferation and migration rates (Pathipati et al., 2011), suggesting that the effects of PRL in neurogenesis may be dose dependent.

The lack of in vitro evidences that support the in vivo observations describing the valuable influence of PRL in hippocampal neurogenesis, may indicate that in vivo, the impact of PRL in this particular neurogenic niche may be mediated by indirect mechanisms (Wagner et al., 2009). As a matter of fact, the promoting of neurogenesis by PRL may be age- and species-dependent, since high levels of PRL during early-age were associated with a reduction in hippocampal neurogenesis in postnatal rats (Lajud et al., 2013), differing from the proliferative effect observed in adulthood mouse (Walker et al., 2012). Furthermore, the negative impact of PRL administration in both hippocampal and olfactory bulb neurogenesis in early life possibly contributes to the development of anxiety behaviors in males, but not females, during adulthood. However, the exact mechanism involved in such observations remained unknown (Lajud et al., 2013). A detailed review regarding the relation between motherhood, maternal experience and neurogenesis, not restricted to PRL-mediated effects, can be consulted elsewhere (Medina and Workman, 2020).

Effects of prolactin in neuroplasticity

Recently, analysis of the proteome of postpartum maternal preoptic area revealed that the common regulators and targets of the significantly altered proteins found between mother and pup-deprived female rats, like AKT, MAPK1 and STAT3, could be traced to PRL. Thus, PRL may be in part responsible for motherhood-associated neuroplasticity in this region (Udvari et al., 2019). In fact, PRL, together with estrogen and progesterone, were considered to be possible intermediaries of the medial preoptic area plasticity observed during pregnancy in rats (Uriarte et al., 2020). In addition, PRL may also be associated with brain plasticity modulation during fatherhood. In mandarin voles, experienced and first-time fathers showed increased spine density and greater dendrite length in the medial prefrontal cortex than non-fathers. This alteration may be explained by the observed increase in PRL levels in both experienced and new fathers (Wang et al., 2018).

Exercise ameliorates the PRL response in chronically stressed mice, improving memory consolidation, through stimulation of the hippocampus (Leem et al., 2019). At the synaptic level, it is believed that PRL stimulates long- and short-term synaptic plasticity in the hippocampus of female mice at reproductive age. The enhancement of synaptic strength was not observed in immature females or male mice, suggesting that sex hormones are necessary to preserve long- and short-term plasticity response to PRL (Zamora-Moratalla and Martin, 2021). High serum PRL levels correlate with enhanced performance in learning and memory tasks possibly associated with the plasticity of the hippocampus in female mice (Moreno-Ruiz et al., 2021).

Prolactin in Neuroprotection and Neurological and Psychiatric Disorders

Neuroprotection

In rodents, PRL protects the hippocampus against glutamate excitotoxicity in the kainic acid model in vivo and in vitro (Rivero-Segura et al., 2017; Anagnostou and Morales, 2019; Ortiz-Pérez et al., 2019). Recently, the detailed mechanisms of PRL-mediated neuroprotection have been extensively reviewed (Molina-Salinas et al., 2021). Briefly, in rodents, parenthood seems to protect the hippocampus against neurodegenerative insults (Anagnostou and Morales, 2019; Cabrera et al., 2009). However, PRL neuroprotective properties were also observed in mice treated with PRL prior to kainic acid insult (Anagnostou et al., 2021). These protective effects were associated with increased AKT phosphorylation (Anagnostou et al., 2021) and ERK1/2 activation (Morales et al., 2014).

The pretreatment of rat hippocampal neuronal cultures with PRL prior to glutamate incubation prevented cell death and mitochondrial dysfunction, and inhibited the increase of intracellular calcium levels, triggered by the excitotoxic insult (Rivero-Segura et al., 2017). On the other hand, vasohinibin, generated by enzymatic cleavage of PRL in the hippocampus, prompts neuronal cell death in cultures of mouse hippocampal primary cells (Aroña et al., 2020). These effects were reversed by the addition of PRL which was able to block the negative effects of vasohinibin, suggesting that PRL action in the hippocampus is complex (Aroña et al., 2020).

Neuroprotective effects of PRL have also been reported in retinal cells. PRL reduces gliosis and favors the expression of survival factors in a rat model of light-induced retinal degeneration (Arnold et al., 2014). Furthermore, increased retinal dysfunction observed with age was correlated with the age-associated decrease of PRLR, and reduction of PRL signaling in the retina (Arnold et al., 2020).

Based on its functions as a neuropeptide, PRL may have an important impact in other neurological disorders. Aside from the potential role as a therapeutic agent in excitotoxicity-mediated diseases like neurodegenerative disorders, as discussed above, the number of studies on the relevance of PRL in neurological disorders is still rather limited. Anew, the relevance of PRL in brain health and disease seems limited. Anew, the relevance of PRL in brain health and disease seems limited. Anew, the relevance of PRL in brain health and disease seems limited. Anew, the relevance of PRL in brain health and disease seems limited.

![Diagram of prolactin-mediated effects in the modulation of adult neurogenesis](Image 526x758 to 559x787)

**Figure 3 | Summary of the main prolactin-mediated effects in the modulation of adult neurogenesis.**

Prolactin (PRL) has been associated with modulatory actions in the two major neurogenic niches in adult brain, the subventricular zone (SVZ) and subgranular zone (SGZ) of the dentate gyrus (DG). In the SVZ, progenitor cells differentiate into interneurons that migrate throughout the rostral migratory stream (RMS) to the olfactory bulb. During the early stage of gestation and lactation, PRL increases the number of proliferating cells in the SVZ and raises the amount of new interneurons in the olfactory bulb. The role of PRL in hippocampal progenitor cells neurogenesis is not as consensual. Some evidences support that PRL favors DG cells proliferation, while others suggest that PRL has neither effect or that negatively impacts hippocampal neurogenesis. In addition, recent evidence suggested that the choroid plexus may also be an alternative source of PRL in the rat brain. However, further studies are necessary to understand if choroid plexus derived PRL has any effects in SVZ neurogenesis. LV: Lateral ventricle.
to be complex, possibly tissue-specific, and once more, apparently dependent on a combination of factors such as age or reproductive stage.

### Brain injury

PRL treatment reduced the cerebral infarct area and edema in a rat cerebral ischemia model, and restored brain glutamate levels and intracellular calcium homeostasis (Vermani et al., 2020). Although PRL administration failed to rescue loss of cortical neurons in hypoxic ischemic juvenile rats, increased PRL immunoreactivity was observed in the injured parietal cortex, and this hormone was associated with glial responses that led to the formation of glial scar (Möderschein et al., 2007). In the human brain, hypoxia- and ischemia-related death cases were associated with increased levels of PRL in the CSF, suggesting that the selective passage of PRL to CSF, possibly mediated by the CP, is augmented in these conditions, possibly to adjust osmotic pressure in the brain (Tani et al., 2018) or by disruption of the BBB associated with stroke (Jiang et al., 2018). Notwithstanding, as there is production of PRL in the CP that can be released to the CSF, the increase of this hormone levels in the CSF found in hypoxia can not be ruled out. On the other hand, PRL was also associated with decrease of the BBB permeability by inducing the expression of tight junction proteins claudin-5 and occludin in primary cultures of bovine brain microvascular endothelial cells (Rosas-Hernandez et al., 2013).

In a prospective cohort study, patients with higher PRL levels during the first year after traumatic brain injury and aneurysmal subarachnoid hemorrhage, tended to perform worst in follow-up cognitive and behavioral tests. In this particular case, the higher PRL levels were considered a consequence of a major pituitary dysfunction reported in these patients (Tölli et al., 2019) rather than a neuroproprotective response mechanism. Many of the putative mechanisms involved in the beneficial actions of PRL in brain injury are linked with neuroprotective properties of PRL, as described above. Although some studies report an increase of PRL levels after brain injury, further research is necessary to investigate if this increase has any protective role, especially in humans.

### Multiple sclerosis

Multiple sclerosis is a CNS autoimmune disorder associated with axonal degeneration and demyelination processes (Zhornitsky et al., 2013). In this regard, PRL is able to induce oligodendrocyte precursor cell proliferation and remyelination of in adult female mice brain during pregnancy (Gregg et al., 2007). Although PRL can be associated with modulation and proinflammatory profiles in immune cells, the combined administration of PRL and interferon-β seems to be beneficial as a short-term treatment of experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis (Zhornitsky et al., 2015). On the other hand, PRL produced by immune cells in the late phases of experimental autoimmune encephalomyelitis mouse model is responsible for the development of persistent neuroinflammation in this pathology (Zhang et al., 2019), which may indicate that despite not being responsible for the onset of multiple sclerosis (Costanza et al., 2013), PRL contributes to the pathological outcome of this disease, as previously reviewed (Zhornitsky et al., 2013).

### Psychiatric disorders and stress

Higher levels of PRL are often observed in patients with depression, stress, and psychopathologies. However, this so-called hyperprolactinemia state is probably a consequence of pharmacological treatment with D2 dopamine receptors blockers and not a cause of psychiatric disorders (Alosaimi et al., 2018). Nonetheless, it has been suggested that higher PRL levels, associated with higher stress rates, in some risk-prone and first-episode psychiatric groups, that had never been treated with antipsychotics, could be associated with emerging psychosis (Ittig et al., 2017; Studerus et al., 2021). This hypothesis lacks the support of robust evidence that directly link higher circulating PRL levels with the onset of mental disorders.

It has been considered that PRL has anxiolytic properties, particularly relevant in the perinatal and postpartum periods (Torner et al., 2002; Larsen and Grattan, 2010; Golub et al., 2016). For instance, female mice exposed to trauma during gestation (exposure to contextual fear and repeated lower levels of PRL during postpartum) and decreased maternal behavior. Furthermore, pups of traumatized mothers, were born smaller and remained smaller throughout life when compared to non-traumatized animals and present anxiety-like behaviors (Golub et al., 2016). Similar results have been reported in humans, where the reduction of PRL levels was also observed in the plasma of mothers with depressive and anxiety symptoms during the perinatal phase, which was associated with reduced social interactive behavior and irritability in newborns (Zhang et al., 2018, 2017). Additionally, augmented brain PRL levels in rats exposed to stress and sleep-deprivation suggest that PRL is also associated with extended REM sleep periods and thus with stress coping mechanisms (Machado et al., 2017).

On the contrary, evidences that PRLR knockdown could be beneficial to the treatment of depression in mice subjected to chronic mild stress have also been reported (Tian et al., 2019). In addition, the administration of exogenous PRL to rats during early-life stages, elicits depressive-like behaviors (Lajud et al., 2013). Also in rats, the administration of bromocriptine, a dopamine receptor agonist widely used to inhibit PRL secretion during early lactation, interrupted with lower peripheral PRL levels and the development of increased anxiety-like behaviors in adult life, whereas the administration of the same compound in the late phase of lactation had the opposite effect (Carvalho et al., 2016). Based on these findings, the effect of PRL in anxiety-like behaviors seems to be age and species dependent.

### Glioblastoma

Some evidence suggest that PRL may also play an important role in the pathology of primary brain tumors. In the past, many studies focused on the pro-tumorigenic role of PRL signaling in breast and pancreatic cancers (Goffin, 2017). Recently, PRL and PRLR expression have been reported in human and rodents glioblastoma cells and induced PRLR overexpression was associated with increased proliferation, migration and chemoresistance of the tumors (Asad et al., 2019). PRLR was found in all samples of human grade II and III gliomas and in glioblastoma, while only 12% of grade II and III gliomas and approximately 30% and glioblastoma samples, expressed PRL mRNA. Furthermore, the long-term patient survival rate was reduced in grade II and III and glioblastoma that expressed PRL (Asad et al., 2019). In addition, within the group of glioblastoma patients with tumors that express PRL, higher levels of PRLR expression were correlated with decreased survival in men. Interestingly, the opposite was observed in women, in which higher levels of PRLR correlated with decreased survival. Remarkably, low levels of PRLR expression were associated with reduced long-term survival rates in grade II and III glioma in men, while no differences were observed in women, which may suggest that PRL and PRLR roles may be dependent of gender and tumor grade (Asad et al., 2019). PRL treatment and PRLR expression in distinct glioblastoma cell lines, were positively correlated with increased invasion capacity of the cells (Alikharus et al., 2016). Overall, the potential of PRL and PRLR as therapeutic targets and/or biomarkers is of particular interest in glioblastoma.

### Concluding Remarks

PRL is a pleiotropic hormone that mediates a considerable diversity of endocrine, autocrine and paracrine actions. Apart from its fundamental in reproduction and lactation, PRL has remarkable actions in the brain, particularly in the development of parental behavior, adapting brain circuits to the necessities imposed by normal parental care that ultimately promote survival of the offspring. PRL brain actions are wide and mediated through the activation of its receptors. Considering the role of PRL in the regulation of neurogenesis, neuropsychology as well as in neuroprotection, it is tempting to suggest that PRL should be investigated for the treatment of neurological disorders, including neurodegenerative diseases. Nonetheless, this approach should be carefully studied since PRL functions are wide and not restricted to a single target.

Despite some developments over the past decade, there is still a considerable lack of scientific evidence regarding the functions of PRL in the brain, especially in humans. It is important to notice that most of the research devoted to the investigation into PRL functions in the brain were performed in rodents and may not be alike in humans. Although in vivo studies using rodents have boosted our knowledge about brain function, the organization and complexity of the brain itself is very distinct between human and rodents. In fact, PRL systems are distinct in rodents and humans. These differences include the expression of different signaling cascades, and the existence of an additional superdistal promoter that modulates the expression of extrapituitary PRL in humans. An effort in the development
of increasingly complex in vitro models that mimic human brain structures, like organoids, could be a practical tool for the study of PRL brain functions. Overall, several controversial issues like the biological relevance of brain extrapituitary PRL remain unanswered. Further studies are necessary to fully understand the dimension of PRL actions in the brain.

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