

RESEARCH ARTICLE

Prolactin enhances hippocampal synaptic plasticity in female mice of reproductive age

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Abstract

Dynamic signaling between the endocrine system (ES) and the nervous system (NS) is essential for brain and body homeostasis. In particular, reciprocal interaction occurs during pregnancy and motherhood that may involve changes in some brain plasticity processes. Prolactin (PRL), a hormone with pleiotropic effects on the NS, promotes maternal behavior and has been linked to modifications in brain circuits during motherhood; however, it is unclear whether PRL may regulate synaptic plasticity. Therefore, the main aim of the present work was to determine the cellular and molecular mechanisms triggered by PRL that regulate synaptic plasticity in the hippocampus. By analyzing extracellular recordings in CA3-CA1 synapses of hippocampal slices, we report that PRL modifies short and long-term synaptic plasticity in female mice of reproductive age, but not in sexually immature females or adult males. This effect is carried out through mechanisms that include participation of GABA_A receptors and activation of the JAK2-mediated signaling pathway. These findings show for the first time how PRL enhances the synaptic strength in hippocampal circuits and that this effect is sexually dimorphic, which would influence complex brain processes in physiological conditions like pregnancy and lactation.

KEYWORDS

hyperprolactinemia, lactancy, LTP, motherhood, PTP, sexual dimorphism

1 | INTRODUCTION

Maternal brain adaptation during pregnancy and lactation is critical to ensure the survival of mammals and plastic changes occur at the circuit and synaptic levels (Horrell, Hickmott, & Saltzman, 2019). In addition, the role of neuroendocrine factors including estrogens, progesterone, oxytocin, vasopressin, prolactin (PRL) and placental lactogen (PL) have been identified during gestation, postpartum, and lactation (Bridges, 2015). In this scenario, PRL (a single-chain polypeptide hormone) and PL play an essential role (Grattan, 2002), since a tight relationship between them exist: High amounts of PL are secreted during the second half of pregnancy, and high serum concentrations of PRL

are found during lactation (Peake, Buckman, Davis, & Standefer, 1983; Robertson & Friesen, 1981). However, the related consequences of PRL at the synaptic level in different brain areas remain unclear.

Beyond classical functions attributed to PRL in lactogenesis, galactopoiesis and reproductive behavior, several lines of evidence indicate that PRL modulates diverse brain processes including neurogenesis (Larsen & Grattan, 2010; Mak et al., 2007; Shingo et al., 2003), neuroprotection (Rivero-Segura et al., 2017), sleep (Machado, Rocha, & Suchecki, 2017; Zhang, Kimura, & Inoué, 1999), and learning & memory (Kinsley et al., 1999; Love et al., 2005; Walker et al., 2012). Clinical evidence obtained during late pregnancy and the early postpartum period shows a significantly lower score on tasks of

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verbal recall and processing speed compared with non-pregnant controls (Henry & Sherwin, 2012). This impairment was associated with high levels of PRL and steroid hormones (Henry & Sherwin, 2012). On the other hand, experimental data indicate that reproductive experience significantly improves spatial reference and working memory (Kinsley et al., 1999; Love et al., 2005; Pawluski, Walker, & Galea, 2006) compared with nulliparous female controls. In addition, PRL null mice exhibit significant hippocampal-dependent learning and memory deficits that are reversed by injection of recombinant PRL into the hippocampus (Walker et al., 2012). Closely related to these findings, morphological studies in the CA1 region of the hippocampus have shown that density and morphology of dendritic spines can be modified by pregnancy and postpartum (Brusco et al., 2008; Kinsley et al., 2006), as well as by reproductive experience (Pawluski & Galea, 2006). Although these effects can be attributed to the increase of PRL in these physiological situations, there have been no studies to date that determine the specific role of this hormone in synaptic plasticity.

Long-term modifications of hippocampal synaptic efficacy are crucial for learning and memory (Martin, Grimwood, & Morris, 2000; Nicoll, 2017; Segal, 2017). However, to date a relationship between PRL and synaptic plasticity in the hippocampus has not been established. Since previous data have suggested that the hippocampus is affected by mothering and neuroendocrine factors such as PRL (Pawluski, Lambert, & Kinsley, 2016), we investigated the synaptic mechanisms that underlie the functional effects of PRL at CA3-CA1 glutamatergic synapses in the CA1 region of the hippocampus. We observed that PRL modulates synaptic plasticity only in female mice of reproductive age. Several mechanisms may be involved in this phenomenon, including participation of GABA_A receptors and the activation of Janus tyrosine kinase 2 (JAK2) and signal transducer and activator of transcription (STAT) proteins signaling. The synergistic action of these events allows for increased of short and long-term potentiation (LTP) in the presence of PRL.

2 | MATERIALS AND METHODS

2.1 | Animals

All procedures were carried out in RjHan/NMRI mice (Janvier Labs, Le Genest Saint Isle, France; $N = 72$) and institutional bioethics committees (Cajal Institute Ethics Committee on Human and Animal Experimentation and CSIC Ethics Committee) approved the experimental protocols in accordance with Spanish (RD 53/2013) and European Communities Council Directives (63/2010/EU).

2.2 | Experimental groups

The experiments were performed in male (2–3 months-of-age; $N = 6$) and intact ($N = 50$) or ovariectomized (OVX, $N = 16$) female mice. Intact female animals were randomly divided into the following

experimental groups: (a) nulliparous females of reproductive age (2–3 months-of-age; $N = 20$) in the diestrus phase, (b) nulliparous female mice (2–3 months-of-age) implanted with an osmotic pump filled with PRL (PRL *op*; to have a continuous release of 150 $\mu\text{g}/\text{day}$ for 7 days; $N = 3$) or vehicle (Vehicle *op*; $N = 3$); (c) immature females (P20–P27; $N = 4$) and (d) sham animals ($N = 20$); see below. On the other hand, OVX mice were treated as follows: (e) OVX mice injected subcutaneously (s.c.) with sesame oil as vehicle, 10 ml/kg (OVX Vh; $N = 9$); and (f) OVX mice injected s.c. with 3 $\mu\text{g}/\text{kg}$ of 17 β -estradiol in sesame oil at a volume of 10 ml/kg (OVX βE ; $N = 7$). Vehicle or 17 β -estradiol was administered in the skinfold at the nape of the back once a day (at 9 a.m.) for seven consecutive days (500 μl), starting 2 weeks after the surgery. In all OVX mice, uterotrophic activity was evaluated establishing a ratio between body and uterine weight.

2.3 | Osmotic pump implant

Animals were anesthetized with 1–3% isoflurane (IsoFlo, Esteve, Barcelona, Spain), which was delivered using a small anesthesia mask connected to a calibrated vaporizer at a flow rate of 1–4 L/min oxygen. The day before surgery, osmotic pumps (Model 2001, Alzet, Cupertino, CA) were filled with PRL or vehicle and placed in sterile 0.9% saline at 37°C overnight. Model 2001 pumps release their contents (200 μl) at a rate of 1 $\mu\text{l}/\text{h}$ continuously for 7 days (150 $\mu\text{g}/\text{day}$ PRL). Mice were anesthetized on a heating pad, a dorsal incision was made in the skin and an osmotic pump was implanted subcutaneously at 9:00 a.m. The skin was closed using suture. Experiments were carried out 7 days after the pump implantations. Delivery was verified by measurement of the residual volume in the pump reservoir after removal.

2.4 | Ovariectomy

Adult female (7–8 weeks-of-age) mice were bilaterally ovariectomized under isoflurane anesthesia on a heating pad (see above). Two dorsal incisions were made in the skin and each ovary was removed through two incisions in the dorsal muscles. The muscle layer and skin were closed using sutures. Sham animals received anesthesia and an incision on the dorsal back, sectioning the skin, and stitching similarly to that of the OVX animals without extirpation of the ovaries. Electrophysiological measurements were performed 3 weeks after surgery. After craniotomy and making the brain slices (see below), postmortem verification of uterus weight confirmed the successful ovariectomy.

2.5 | Electrophysiology

Detailed methods of most of the procedures have been described previously (Lao-Peregrín et al., 2017; Martín & Buño, 2003). Briefly, transverse slices (350–400 μm thick) from the mouse brains were cut with a vibratome (VT1200S, Leica Microsystems, Nussloch,

Deutschland) and incubated for >1 hr at room temperature (21–24°C) in artificial cerebrospinal fluid (aCSF) that contained 124 mM NaCl, 2.69 mM KCl, 1.25 mM KH₂PO₄, 2 mM MgSO₄, 26 mM NaHCO₃, 2 mM CaCl₂ and 10 mM glucose, and gassed with a 95% O₂/5% CO₂ mixture at pH 7.3–7.4. Individual slices were transferred to an immersion recording chamber and perfused with oxygenated aCSF at 2 ml/min warmed at near physiological temperature (30 ± 2°C). Field postsynaptic potentials (fEPSPs) were recorded via a carbon fiber microelectrode (Carbostar-1, Kation Scientific, Minneapolis, MN) placed in the stratum radiatum of the CA1 pyramidal layer. Evoked fEPSPs were elicited by stimulation of the Schaffer collateral fibers (SCs) with an extracellular bipolar tungsten electrode via a 2,100 isolated pulse stimulator (A-M Systems, Inc., Carlsborg, WA) that was set to deliver monophasic currents of 50 μs duration. At the beginning of each experiment, basal synaptic transmission was analyzed by applying isolating stimuli of increasing intensity to reach a maximal fEPSP response. For LTP experiments, the stimulus intensity was adjusted to elicit 50% of the maximum response signal and kept constant throughout the experiment. Data were stored through an acquisition system PowerLab 4/26 (AD Instruments, Bella Vista, Australia) and the software Scope (AD Instruments) was used to display fEPSP and measurements of fEPSP slopes. After recording stable baseline responses for 30 min, LTP was induced by a single train of theta burst stimulation (TBS; 5 bursts of 5 pulses at 100 Hz, with an interval of 200 ms between bursts). Potentiation was measured for 1 hr after LTP induction at 0.033 Hz. Changes in the fEPSP slope were calculated in relation to the baseline fEPSP responses during the last 10 min before TBS, and the time course of LTP values was then normalized to this baseline. The mean slope of the fEPSP during the first 5 min after LTP-inducing TBS was used to measure post-TBS potentiation (PTP).

2.6 | Drugs

Sheep PRL and picrotoxin (PTX) were purchased from Sigma (St Louis, MO) and AG490 from Tocris Cookson (Bristol, UK). Drugs were prepared as stock solutions, stored frozen in the dark, and diluted to final concentration immediately before use. Sheep PRL (44.5 μM) was prepared in milli-Q water while AG490 (100 mM) and 17β-estradiol (50 mg/ml) were prepared in dimethyl sulfoxide and dissolved in sesame oil for s.c. injection. Drugs were dissolved in oxygenated aCSF at the desired concentration immediately before use. Slices were incubated (20 min) in sheep PRL before being transferred to the immersion chamber, in which PRL was present for the entire recording period at the same concentration.

2.7 | Data analyses

Control LTP measurements were performed alternately with experimental tests (i.e., PRL perfusion with and without treatment). The slices were discarded if the recording of baseline fEPSP slope was not stable for 30 min and/or if the reproducible fEPSP amplitude varied

>20% from initial values. In each experiment, *N* represents the number of animals whereas *n* represents the number of slices. The value of the weight of the uterus was normalized to correct for variability between animals for the same age range, by establishing a ratio between body weight and weight of the uterus [(g uterus/g animal) × 100]. All data are expressed as mean ± SEM. In order to choose the correct statistical test/analysis, we first studied whether the distribution of the data were normal (parametric data) or not (non-parametric data) by using the Shapiro–Wilk test. To study differences induced in the same slice (i.e., Basal vs. PTP, Basal vs. LTP) we used a paired *t* test when the data showed a normal distribution or Wilcoxon matched-pairs signed rank test when the distribution was not normal. To establish differences between treatments or experimental conditions, we used unpaired *t* test to compare between two experimental groups or ANOVA to compare between more than two groups and Dunnett's multiple comparisons test. For non-parametric data, we used Mann Whitney (two groups) or Kruskal–Wallis tests and Dunn's multiple comparisons test (more than two groups). Statistical analysis was performed by SigmaPlot (Systat SoftwarE, Inc., San José, CA) and GraphPad Prism v 6.0 (GraphPad Software, San Diego).

3 | RESULTS

To investigate whether PRL modified synaptic plasticity, we studied its effect on glutamatergic CA3–CA1 synapses from mouse hippocampal slices (Figure 1a). First, we analyzed nulliparous female mice of reproductive age (2–3 months, diestrus phase) using a TBS conditioning stimulus to induce a sustained increase in synaptic strength, so-called LTP. In the presence of 200 nM PRL, TBS induced a statistically significant increase in fEPSP slope at 5 min (PTP) compared to control conditions without PRL (Figure 1b,c,e). Such potentiation was sustained 60 min after LTP induction (Figure 1b,d,f). Since PRL levels in the brain areas are variable according to physiological conditions (Ben-Jonathan, LaPensee, & LaPensee, 2008; Freeman, Kanyicska, Lerant, & Nagy, 2000), we further determined the effect of different concentrations of PRL added to the hippocampal slices. The statistically significant difference in PTP and LTP was maintained at 400 nM PRL with respect to control, achieving similar values to 200 nM PRL (Figure 1e,f). By contrast, fEPSP values obtained at 20 nM PRL were indistinguishable from their control values (Figure 1e,f). Interestingly, the PRL-induced increase in synaptic strength in nulliparous females of reproductive age was not observed in either sexually immature females between P20 and P27 (Figure 2a–c) or in adult male animals (Figure 2d–f). Therefore, present results indicate that PRL modulates short (PTP) and long-term (LTP) synaptic plasticity specifically in the hippocampus from females of reproductive age mice.

Perfusion of neuroactive substances in brain slices is a well-established and common very extended experimental model that allows us to adequately control many physiological variables. However, it is possible that the dose needed to evoke an effect *ex vivo* is different from the physiological serum values *in vivo*. Therefore, our next step was to implant nulliparous female mice with an

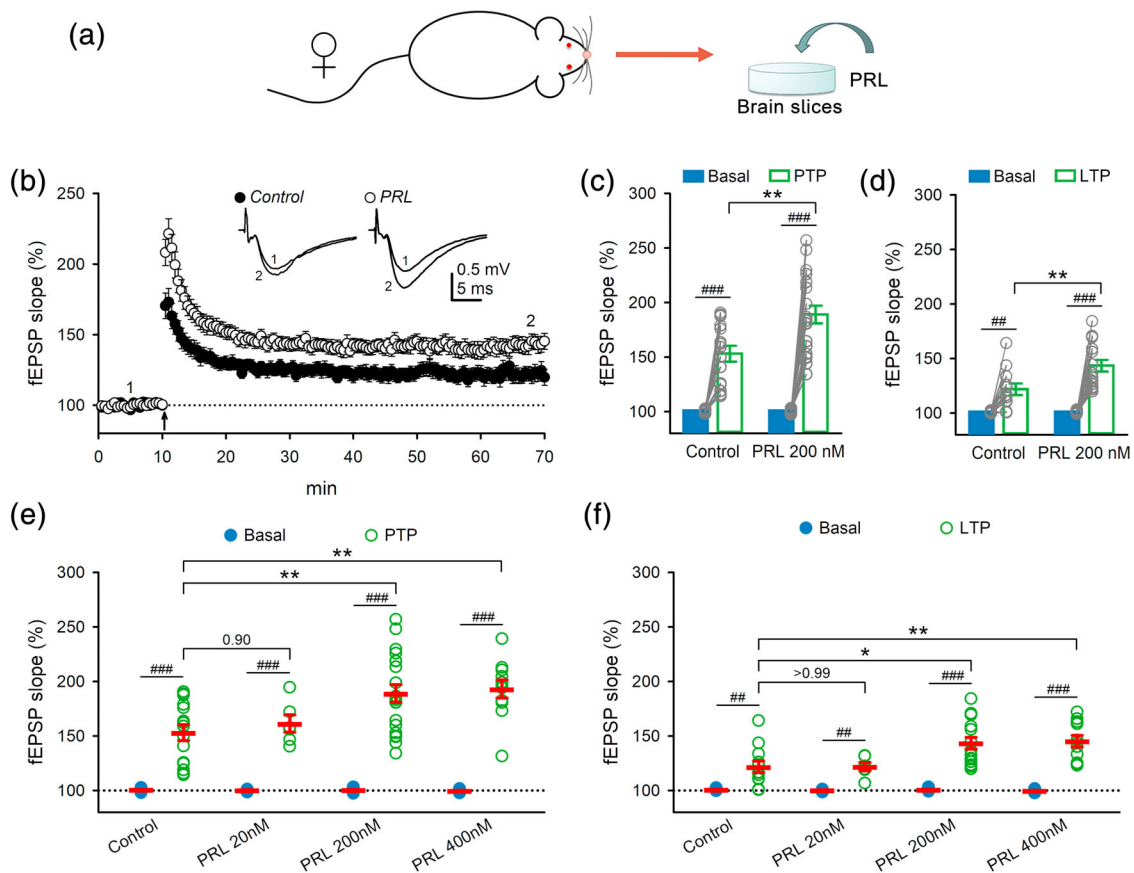


FIGURE 1 Prolactin improves hippocampal synaptic plasticity in females of reproductive age. (a) Experimental design. Brain slices were prepared from female mice at 2–3 months of age, and prolactin (PRL) was added for 20 min before measurements of electrophysiology (b) In the presence of 200 nM PRL (empty circles, $n = 20$, $N = 9$) magnitude of fEPSP was significantly enhanced compared to controls (filled circles, $n = 15$, $N = 7$). Insets of traces in the plots represent average fEPSPs recorded during periods indicated by corresponding numbers in the graph (1 and 2). (c) Percentage of fEPSP slope (taken from (b)) before (basal) and after TBS at 5 min (PTP) in control condition ($153.10 \pm 7.26\%$; $n = 15$, $N = 7$) compared in the presence of 200 nM PRL ($188.94 \pm 8.06\%$; $n = 20$, $N = 9$; unpaired t test: $t_{[33]} = 3.188$; $**p = .0031$). Statistical differences with respect to basal state were established with paired t test (control, $###p < .001$, $t_{[14]} = 7.260$; PRL, $###p < .001$, $t_{[19]} = 10.97$). (d) Percentage of fEPSP slope (taken from (b)) before (basal) and after TBS at 60 min (LTP) in control condition and in the presence of 200 nM PRL ($143.4 \pm 5.36\%$; $n = 16$, $N = 9$ for PRL vs. $121.82 \pm 5.26\%$; $n = 12$, $N = 6$ for control; Mann–Whitney test: $U_{[12,16]} = 37$; $**p = .0052$). Statistical differences with respect to basal state were established with paired t test for control ($##p = .002$, $t_{[11]} = 4.033$) and Wilcoxon matched-pairs test for PRL ($###p < .001$, $W = 136$). (e) Same as (c) but at 20 nM PRL (PTP: $161.28 \pm 7.99\%$; $n = 6$, $N = 3$ for PRL vs. $153.1 \pm 7.26\%$; $n = 15$, $N = 7$ for control; ANOVA: $F_{[3,48]} = 5.576$; $p = .0023$; Dunnett's test: $p = .9023$) and 400 nM (PTP: $193.11 \pm 8.23\%$; $n = 11$, $N = 4$ for PRL vs. $153.1 \pm 7.26\%$; $n = 15$, $N = 7$ for control; ANOVA: $F_{[3,48]} = 5.576$; $p = .0023$; Dunnett's test: $p = .0053$). Statistical differences with respect to basal state were established with paired t test (20 nM, $###p < .001$, $t_{[5]} = 7.757$; 400 nM, $###p < .001$, $t_{[10]} = 11.34$). (f) Same as (d) but at 20 nM PRL (LTP: $121.91 \pm 3.83\%$; $n = 6$, $N = 3$ for PRL vs. $121.82 \pm 5.26\%$; $n = 12$, $N = 6$ for control; Kruskal–Wallis test: $H_{[3]} = 13.937$; $p = .003$; Dunn's test: $p > .9999$) and 400 nM (LTP: $145.22 \pm 5.41\%$; $n = 11$, $N = 4$ for PRL vs. $121.82 \pm 5.26\%$; $n = 12$, $N = 6$ for control; Kruskal–Wallis test: $H_{[3]} = 13.937$; $p = .003$; Dunn's test: $**p = .0095$). Statistical differences with respect to basal state were established with paired t test (20 nM, $##p = .0018$, $t_{[5]} = 6.005$; 400 nM, $###p < .001$, $t_{[10]} = 8.365$) [Color figure can be viewed at wileyonlinelibrary.com]

osmotic pump (150 $\mu\text{g/day}$ for 7 days; Figure 3a) in order to achieve high serum PRL levels (≈ 260 ng/ml, Sonigo et al., 2012), like those present in lactating mice (≈ 360 ng/ml, Brown, Herbison, & Grattan, 2011; ≈ 100 ng/ml, Guillou et al., 2015). Chronic PRL treatment via osmotic pump induced a statistically significant increase of fEPSP with respect to vehicle controls after induction and during maintenance of LTP (Figure 3b–d), in line with our data obtained after incubation with 200 nM PRL. This result supports that high PRL concentrations can induce changes in hippocampal synaptic plasticity.

Estrogen from the ovaries is one of the most important regulators of PRL secretion in different physiological states (Grattan, 2015). Consequently, in order to determine the influence of gonadal function on the synaptic changes mediated by PRL described above, we next performed a set of experiments on OVX female mice (Figure 4a; see Experimental Procedures). As is well known, ovariectomy significantly reduces the uterus weight ratio in OVX compared with sham, and this effect can be reversed by 17β -estradiol treatment (Figure 4b). Therefore, we used the uterus weight ratio to confirm the success of ovariectomy and hormonal treatment in the following experiments.

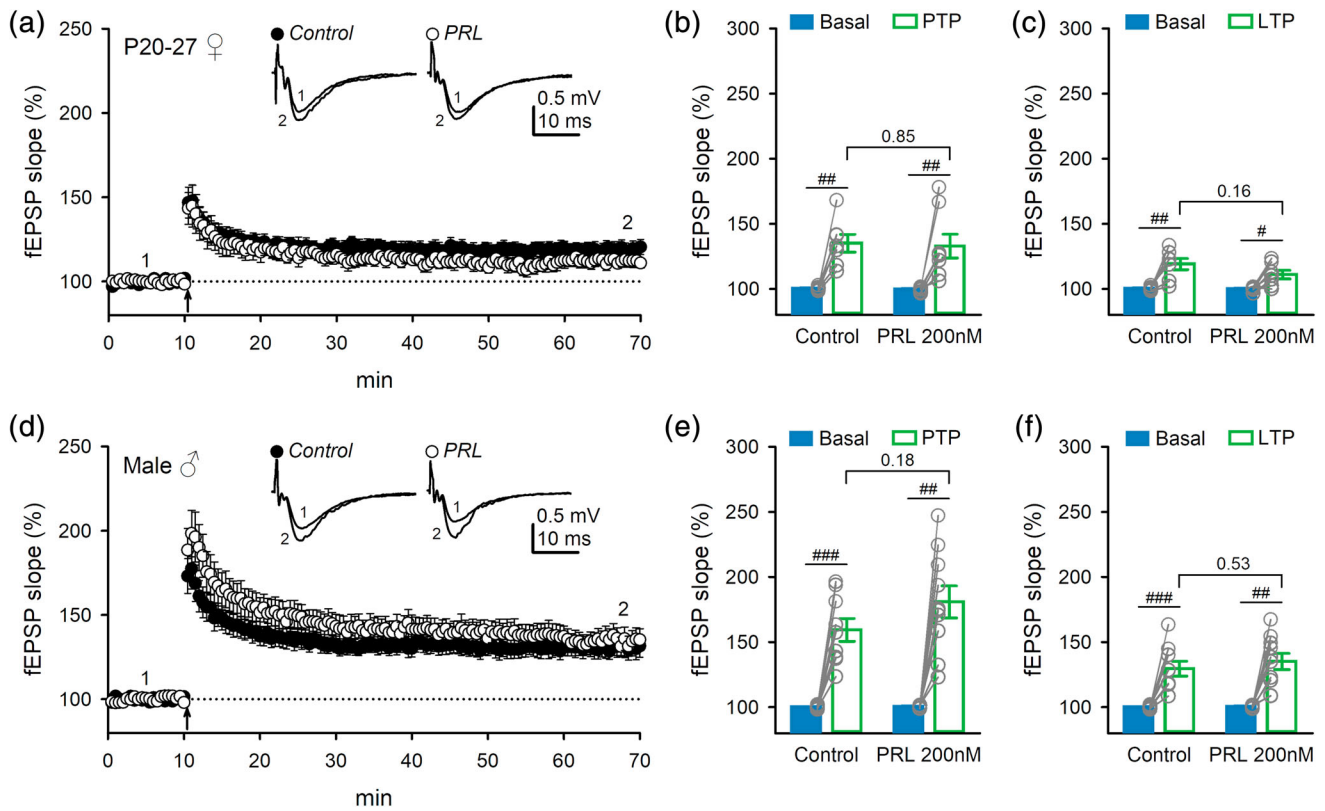


FIGURE 2 Prolactin's effect on hippocampal synaptic plasticity is exclusive to females of reproductive age. (a) An increase in magnitude of PTP and LTP in the presence of prolactin (PRL) was not observed in sexually immature female mice. Trace insets show representative fEPSPs (1 and 2). (b) Percentage of PTP in control condition and in the presence of 200 nM PRL (PTP: 132.87 ± 9.10 ; $n = 8$, $N = 4$ for PRL vs. 135.06 ± 6.83 %; $n = 7$, $N = 4$ for control; unpaired t test: $t_{[13]} = 0.1882$; $p = .8536$). Significant differences with respect to basal state were established with paired t test (control, $^{##}p = .002$, $t_{[6]} = 5.193$; PRL, $^{###}p = .0078$, $t_{[7]} = 3.685$). (c) Percentage of LTP in control condition and in the presence of 200 nM PRL (LTP: 110.96 ± 3.33 %; $n = 7$, $N = 4$ for PRL vs. 119.08 ± 4.31 %; $n = 7$, $N = 4$ for control; unpaired t test: $t_{[12]} = 1.490$; $p = .162$). Significant differences with respect to basal state were established with paired t -test (control, $^{##}p = .0034$, $t_{[6]} = 4.674$; PRL, $^{#}p = .017$, $t_{[6]} = 3.276$). (d) No statistically significant differences were found in slices of males of reproductive age in the presence of PRL. Trace insets show representative fEPSPs (1 and 2). (e) Percentage of PTP in control condition and in the presence of 200 nM PRL (PTP: 180.83 ± 12.32 %; $n = 10$, $N = 4$ for PRL vs. 159.3 ± 8.77 %; $n = 9$, $N = 4$ for control; unpaired t test: $t_{[17]} = 1.394$; $p = .1813$). Significant differences with respect to basal state were established with paired t -test for control ($^{###}p < .001$, $t_{[8]} = 6.814$) and Wilcoxon matched-pairs test for PRL ($^{##}p = .002$, $W = 55$). (f) Percentage of LTP in control condition and in the presence of 200 nM PRL (LTP: 135.03 ± 6.22 %; $n = 10$, $N = 4$ for PRL vs. 129.59 ± 5.72 %; $n = 9$, $N = 4$ for control; unpaired t test: $t_{[17]} = 0.6386$; $p = .5316$). Significant differences with respect to basal state were established with paired t -test for control ($^{###}p < .001$, $t_{[8]} = 5.186$) and Wilcoxon matched-pairs test for PRL ($^{##}p = .002$, $W = 55$) [Color figure can be viewed at wileyonlinelibrary.com]

Electrophysiological recordings from hippocampal slices of OVX mice did not reveal a significant difference in the ability of TBS to induce PTP or LTP after incubation with PRL (Figure 4c–e), in line with our previous findings in immature females. Interestingly, a significant difference in PTP and LTP was maintained in the presence of PRL in animals treated with 17β -estradiol with respect to OVX mice treated with vehicle in the absence of PRL (Figure 4c–e). On the other hand, no significant changes in PTP or LTP were found in brain slices from OVX mice treated with vehicle compared with those treated with 17β -estradiol, either in the absence or presence of PRL (Figure 4e). Taken together, the present data suggest that integrity of gonadal function is required to maintain the effect of PRL on short- and long-term synaptic plasticity.

To explore possible mechanisms underlying the improvement in synaptic plasticity induced by PRL, we first examined basal synaptic transmission in the CA1 region in intact female mice of reproductive

age by applying isolated stimuli of increasing intensity to the SCs. Perfusion of PRL (200 nM) had no effect on basal fEPSP for a wide range of stimulation intensities (Figure 5a,b; see statistics in Table S1). Likewise, measurements of the fiber volley amplitude, which indicates the activation of a compound action potential from SCs axons, were similar in control conditions and in the presence of PRL in the bath (Figure 5c; see statistics in Table S1). Additionally, no differences were found in the input/output coupling (Figure 5d). Collectively, these results indicate that PRL does not alter basal synaptic transmission at CA3-CA1 contacts, but indeed, it does boost synaptic plasticity processes.

In order to explore whether the PRL effects on LTP can be attributed to decreased inhibition, increased excitation or an excitatory/inhibitory imbalance in the CA3-CA1 synapses, we tested the role of γ -amino-butyric acid (GABA) type A ($GABA_A$) receptor-mediated inhibitory synaptic transmission in modulation of synaptic plasticity by PRL. TBS was applied to control and after PRL incubation in the

presence of the GABA_A antagonist PTX (100 μ M) in intact female mice of reproductive age. In this condition, blockage of GABA_A receptors (GABA_AR) prevented PRL-dependent enhancement of fEPSP (Figure 6a–c), demonstrating that decreased inhibition plays a significant role in the enhancement of LTP induced by PRL. Interestingly, significant differences were found in PTP (but not in LTP) when comparing slices treated with PTX in the presence of PRL and control condition (Figure 6d,e; Control and PRL 200 nM are data taken from Figure 1). In addition, no statistically significant differences were found in PTP or LTP when comparing slices incubated in PRL with or without PTX (Figure 6d,e; Control and PRL 200 nM are data taken from Figure 1), reinforcing the importance of the GABAergic system in PRL's effect on synaptic plasticity.

The binding of PRL with its receptor (PRL_R) activates different intracellular signaling pathways as well as phosphorylation of the receptor-associated JAK2 (Freeman et al., 2000). Downstream, this kinase phosphorylates STAT (DaSilva et al., 1996) proteins. Since JAK2/STAT signals have been reported to play an important role in synaptic plasticity (Nicolas et al., 2012) and memory (Chiba et al., 2009), we hypothesized that LTP enhancement by PRL is mediated by this pathway. To address this possibility, hippocampal slices

from intact female mice of reproductive age were treated with the JAK inhibitor AG490 (Chiba et al., 2009; Nicolas et al., 2012). We found that perfusion with AG490 (10 μ M) does not modify basal fEPSP slope (in agreement with previous observations; Nicolas et al., 2012), and no significant differences in the magnitude of PTP or LTP were observed in the presence of PRL (Figure 7a–c). Therefore, inhibition of JAK signaling prevented the enhancement of PTP or LTP induced by PRL, reaching similar values to those obtained under control conditions (Figure 7d,e, Control and PRL 200 nM are data taken from Figure 1). These results reveal that PRL requires the JAK/STAT signaling pathway to increase short and long-term synaptic plasticity in CA3-CA1 hippocampal synapses.

4 | DISCUSSION

Strong clinical and experimental evidence support that during pregnancy, delivery and lactation, modifications in hormonal levels generate structural and functional brain changes that modify the behavioral responses of females and induce a high state of maternal responsiveness (Bridges, 2015; Horrell et al., 2019). Plastic changes at circuit and synaptic levels in the NS may underlie these new adaptive behaviors, which involve dendritic growth, increases in spine density and

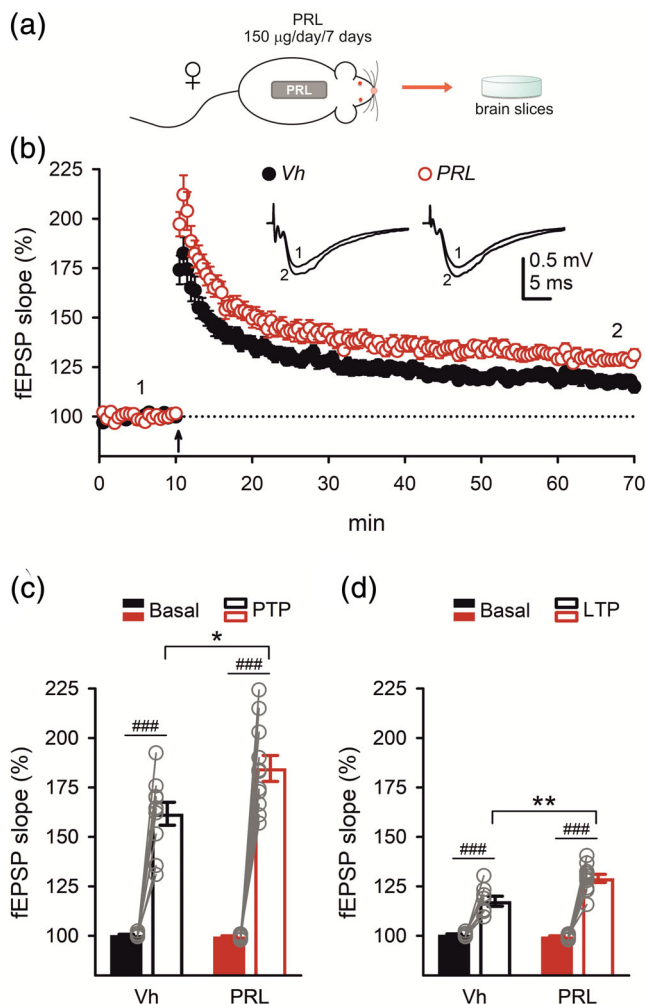


FIGURE 3 Systemic treatment with prolactin is sufficient to improve synaptic plasticity in the hippocampus. (a) Experimental design. Brain slices were prepared from female mice at 2–3 months-of-age implanted with an osmotic pump (150 μ g/day for 7 days). (b) Time course of mean fEPSP slope in hippocampus slices from animals treated with vehicle (Vh, filled circle, *n* = 10, *N* = 3) and prolactin administered by osmotic pump (PRL, open circle, *n* = 11, *N* = 3) in basal conditions and following theta burst stimulation of SC (arrow). Trace insets show representative fEPSPs recorded during periods indicated by corresponding numbers in the graphic (1 and 2). (c) Summary data (taken from (b)) showing mean fEPSP slopes in hippocampal slices before (Basal) and after 5 min (PTP) application of TBS in control condition (Vh) and after treatment with PRL (PRL). Significant differences with respect to basal state were established with paired *t* test (Vh: 100.45 \pm 0.29%, *n* = 10, *N* = 3 for basal vs. 161.70 \pm 5.80%, *n* = 10, *N* = 3 for PTP; ###*p* < .001, *t*_[9] = 10.43; PRL: 99.72 \pm 0.28%, *n* = 11, *N* = 3 for basal vs. 184.58 \pm 6.57%, *n* = 11, *N* = 3 for PTP; ###*p* < .001; *t*_[10] = 12.77). Significant differences between experimental groups were established with unpaired *t* test (161.67 \pm 5.80%, *n* = 10, *N* = 3 for Vh vs. 184.58 \pm 6.57%, *n* = 11, *N* = 3 for PRL; **p* = .0181, *t*_[19] = 2.587). (d) Summary data (taken from (b)) showing mean fEPSP slopes in hippocampal slices before (basal) and after 60 min (LTP) application of TBS in control condition (Vh) and treated with PRL (PRL). Significant differences with respect to basal state were established with paired *t* test (Vh: 100.62 \pm 0.34%, *n* = 8, *N* = 3 for basal vs. 117.47 \pm 2.5%, *n* = 8, *N* = 3 for LTP; ###*p* < .001, *t*_[7] = 6.455; PRL: 99.72 \pm 0.28%, *n* = 11, *N* = 3 for basal vs. 129.05 \pm 2.02%, *n* = 11, *N* = 3 for LTP; ###*p* < .001; *t*_[10] = 14.06). Significant differences between experimental groups were established with unpaired *t* test (117.47 \pm 2.53%, *n* = 8, *N* = 3 for Vh vs. 129.05 \pm 2.02%, *n* = 11, *N* = 3 for PRL; ***p* = .0021, *t*_[17] = 3.616) [Color figure can be viewed at wileyonlinelibrary.com]

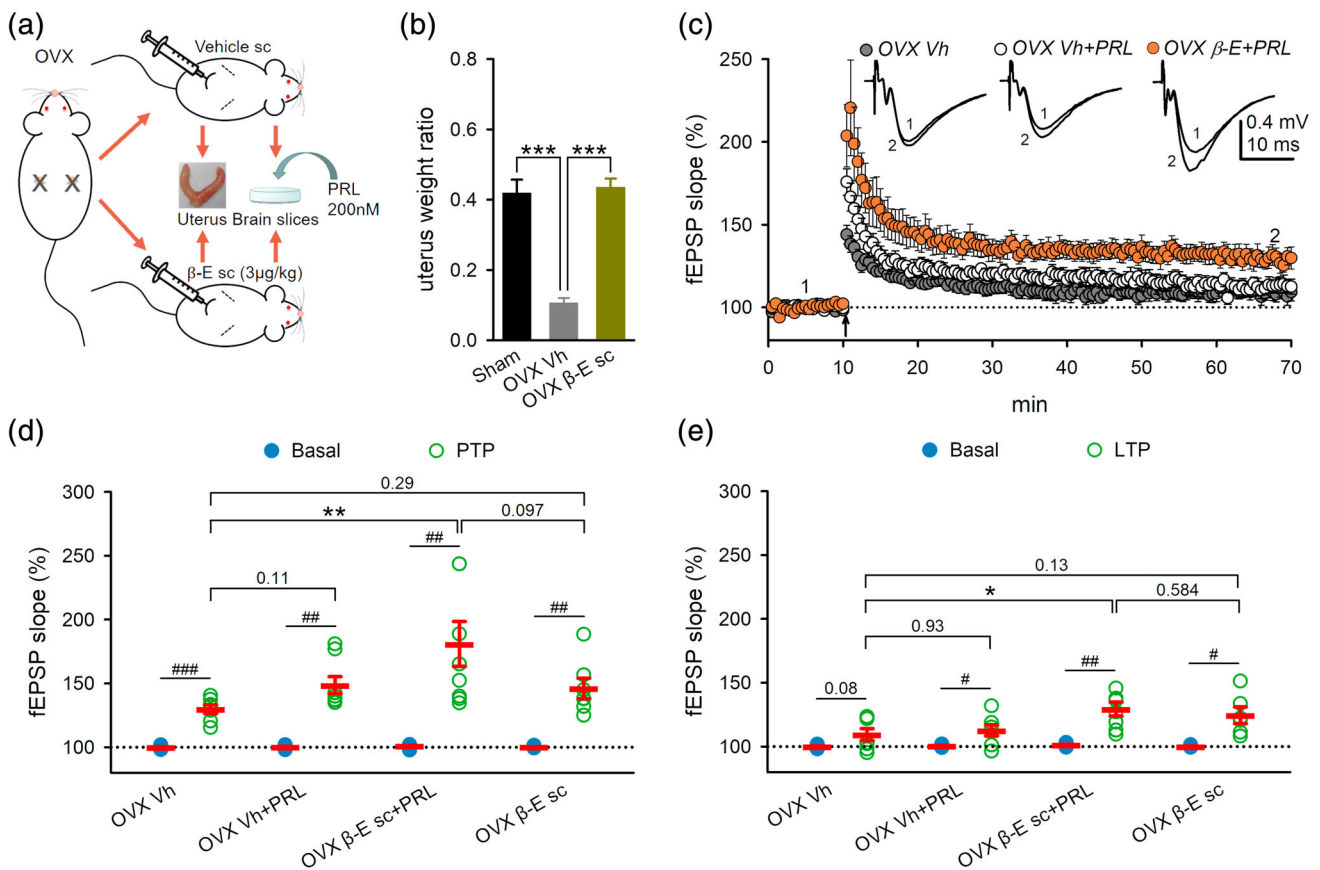


FIGURE 4 Prolactin-dependent changes in hippocampal synaptic plasticity require the integrity of gonadal function. (a) Scheme showing the experimental design. Ovariectomized mice at 7–8 weeks of age were injected or not 2 weeks after the surgery with 17β -estradiol s.c., and 7 days later euthanized for collection of brains and comparison of uterus weight. Brain slices were prepared and incubated with 200 nM prolactin (PRL) for 20 min before electrophysiology measurements (b) Average of uterus weight ratio in sham ($N = 20$), OVX mice treated with vehicle (OVX Vh; $N = 9$), and after s.c. administration of 17β -estradiol (OVX β -E sc; $N = 7$). The difference between the groups was statistically significant (OVX Vh: 0.107 ± 0.013 ; $N = 9$ vs. sham: 0.419 ± 0.038 ; $N = 20$; Kruskal-Wallis test, $H_{[2]} = 20.15$; $p < .001$; Dunn's test; $***p < .001$ and OVX Vh vs. OVX β -E sc: 0.435 ± 0.025 ; $N = 7$; Dunn's test; $***p < .001$) confirming the success of ovariectomy and the estrogen treatment. (c) Summary data showing the time course of mean fEPSP slope in OVX Vh animals (grey filled circle; $n = 7$, $N = 4$), in the presence of 200 nM of PRL (open circle; $n = 8$; $N = 4$), or treated with 17β -estradiol for 7 days and incubated in 200 nM PRL (orange filled circle; $n = 7$, $N = 4$), in basal conditions and following induction of LTP (arrow). Trace insets show representative fEPSPs (1 and 2). (d) Percentage of PTP basal and after TBS in OVX mice (OVX Vh; $n = 7$, $N = 4$), in the presence of 200 nM PRL (OVX Vh + PRL; $n = 8$, $N = 4$), after administration of 17β -estradiol in the presence of 200 nM PRL (OVX β -E sc. + PRL; $n = 7$, $N = 4$) and after treatment with 17β -estradiol (OVX β -E sc; $n = 7$, $N = 4$) for 7 days (Kruskal-Wallis test: $H_{[3]} = 10.35$, $p = .0158^*$; OVX Vh vs. OVX Vh + PRL, Dunn's test, $p = .1087$; OVX Vh vs. OVX β -E s.c. + PRL, Dunn's test, $**p = .0046$; OVX β -E s.c. + PRL vs. OVX β -E sc, unpaired t test: $t_{[12]} = 1.8$, $p = .097$; OVX Vh vs. OVX β -E sc, Dunn's test: $p = .29$). Significant differences with respect to basal state were established with paired t test (OVX Vh: $###p < .001$, $t_{[6]} = 8.504$; OVX β -E s.c. + PRL: $##p = .0036$, $t_{[6]} = 4.620$; OVX β -E s.c.: $##p = .0013$, $t_{[6]} = 5.655$) and Wilcoxon matched-pairs test (OVX Vh + PRL: $##p = .0078$, $W = 36$). (e) Same as (d) but for LTP (ANOVA test: $F_{[3,22]} = 3.269$, $p = .0405$; OVX Vh vs. OVX Vh + PRL, Dunnett's test, $p = .9338$; OVX Vh vs. OVX β -E s.c. + PRL, Dunnett's test, $*p = .0345$; OVX Vh vs. OVX β -E, Dunnett's test, $p = .1286$; OVX β -E s.c. + PRL vs. OVX β -E sc, unpaired t test: $t_{[10]} = 0.566$; $p = .584$). Significant differences with respect to basal state were established with paired t test (OVX Vh: $p = .0819$, $t_{[6]} = 2.087$; OVX Vh + PRL: $#p = .0404$, $t_{[6]} = 2.605$; OVX β -E s.c. + PRL: $##p = .0029$, $t_{[5]} = 5.410$; OVX β -E s.c.: $#p = .0125$, $t_{[5]} = 3.811$) [Color figure can be viewed at wileyonlinelibrary.com]

modifications of synaptic strength in pre-existing connections; all of these results in an extensive remodeling of neural networks (Pascual-Leone, Amedi, Fregni, & Merabet, 2005). The hippocampus is a key brain area involved in several physiological behaviors, such as spatial information acquisition, retrieval and consolidation, and storage of memory (Colgin, Moser, & Moser, 2008; Martin et al., 2000; Nicoll, 2017; Segal, 2017), but the contribution of PRL to synaptic plasticity regulation in the hippocampus is unknown. Here, we

demonstrate for the first time that PRL modifies short and long-term synaptic plasticity in the hippocampus in female mice of reproductive age. PRL levels in brain areas depend on species and sex, and vary according to physiological conditions such as reproductive cycle, gestation and lactation (Ben-Jonathan et al., 2008; Freeman et al., 2000). Particularly in mice, PRL secretion patterns show twice daily PRL surges during early pregnancy that are suppressed during mid-pregnancy (Phillipps, Yip, & Grattan, 2020). In late pregnancy,

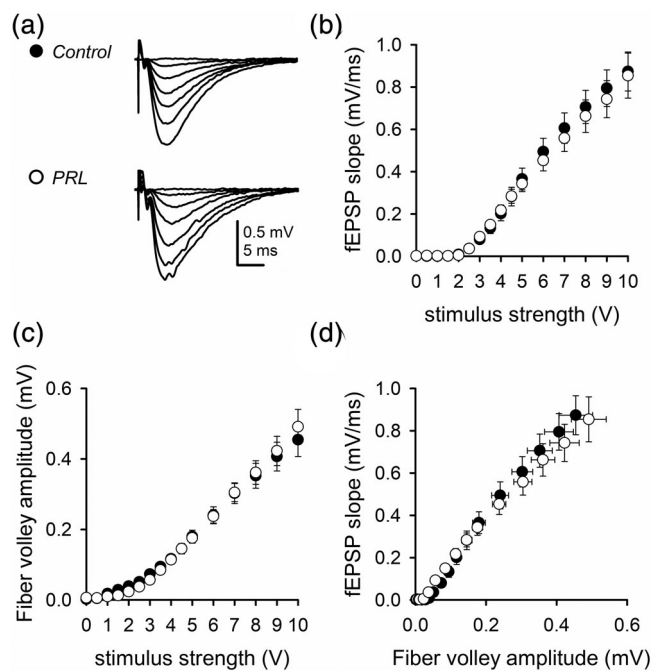


FIGURE 5 Prolactin does not modify the basal synaptic transmission at CA1 synapses. (a) Representative fEPSPs recorded in the stratum radiatum and evoked by stimulation of the SC pathway with different intensities in control (top) and in the presence of 200 nM prolactin (PRL; bottom). (b) fEPSP slopes are comparable between control (filled circle, $n = 20$, $N = 11$) and after treatment with PRL (open circle; $n = 16$, $N = 7$) for a given range of stimulus intensities. (c) Fiber volley amplitudes are similar between experimental conditions (control, filled circle, $n = 20$, $N = 11$; PRL, open circle, $n = 16$, $N = 7$) for a given range of stimulus intensities. (d) Input/output relationships for control (filled circle, $n = 20$, $N = 11$) and in the presence of PRL (open circle, $n = 16$, $N = 7$)

PRL levels rapidly increase and remain high during lactation, with evident rises in PRL levels in response to suckling (Phillipps et al., 2020) which then decline gradually (Guillou et al., 2015). We showed that a rise in serum PRL levels after treatment (using minipumps) induces a statistically significant increase in PTP and LTP magnitude, compared with vehicle. Previous observations have indicated that serum PRL concentrations reach high values after minipump treatment (≈ 260 ng/ml, Sonigo et al., 2012), similar to the levels present during early pregnancy and lactation in mice (i.e., ≈ 360 ng/ml, Brown et al., 2011; ≈ 100 ng/ml, Guillou et al., 2015). Therefore, it was expected that in our experiment we would reach similar values. Complementary to these findings, we determined the effect of different concentrations of PRL by its direct addition to the slice bath. In these circumstances, we observed an effect of PRL on synaptic plasticity down to 200 nM (≈ 5 μ g/ml). However, this concentration is not surprising since experimental conditions (chronic minipump PRL treatment in vivo vs. acute PRL perfusion ex vivo) are very different. In line with our findings, in hypothalamus slices a concentration near 200 nM of PRL is necessary to switch tuberoinfundibular dopamine neuron discharge from phasic to tonic (Lyons, Hellysaz, & Broberger, 2012), or for activation of medial preoptic area galanin

neurons (Stagkourakis et al., 2020). We conclude that acute application of PRL to hippocampal slices at similar concentrations should be a suitable condition to induce changes in hippocampal synaptic plasticity.

Short-term forms of synaptic plasticity are essential to improve the computational capacity of local circuits in the network, allowing the system to modulate its activity on various time scales. PTP is an enhancement of transmitter release on a minute time scale (3 to 5). Other forms of short-term synaptic plasticity such as depression or facilitation have a faster time scale, in the range of milliseconds (Abbott & Regehr, 2004; Zucker & Regehr, 2002). Since the present results showed that PRL enhances PTP it is feasible that this hormone could modify synaptic plasticity on a short time scale in order to improve the computational capacity of the system, that is, filtering information flow across the synapse (Fortune & Rose, 2001).

LTP is one of the most studied models of the cellular mechanisms underlying some forms of learning and memory (Bliss & Collingridge, 1993; Bliss & Lømo, 1973; Mayford, Siegelbaum, & Kandel, 2012). Previous studies have shown that motherhood increase LTP in the hippocampus that can persist after the cessation of lactation (Lemaire et al., 2006; Tomizawa et al., 2003) and the present results reveal that PRL contributes to regulation of synaptic strength in the hippocampus. LTP has an early phase independent of protein synthesis (E-LTP; 1 to 3 hr), and a late phase (L-LTP; lasts at least 24 hr) which involves activation of transcription factors and protein synthesis (Kandel, 2001). Our study has focused on the early phase of LTP. Previous clinical and experimental investigations with oxytocin, a hormone involved in the birth process and milk ejection, showed changes in early and late LTP (Lemaire et al., 2006; Tomizawa et al., 2003) in the hippocampus after weaning, underlining the importance of this hormone for synaptic plasticity (Tomizawa et al., 2003). Oxytocin is able to prolong LTP without modifying the basal synaptic transmission (Tomizawa et al., 2003), in line with the present results showing that PRL increased LTP but did not modify basal neurotransmission at CA3-CA1 synaptic contacts. Our data uncover the role of PRL in functional synaptic changes, showing its contribution to regulate long-term plasticity in the hippocampus. Interestingly, PRL induces inhibition of oxytocin neurons in pregnant rats, but this effect was lost during lactation (Augustine et al., 2017). Therefore, it is possible that both oxytocin and PRL act cooperatively by modifying hippocampal function during relevant physiological conditions such as lactation.

The sex differences in PRL-mediated effects on synaptic plasticity and learning and memory in the hippocampus are not well-established. Studies performed in females and males from PRL null mice showed a reduced performance of hippocampal-dependent learning tasks that was prevented by intra-hippocampal injection of PRL, with no distinction between the sexes (Walker et al., 2012). Here, we have demonstrated that enhancement of hippocampal LTP by PRL is sexually dimorphic, because no changes were observed in male mice. In addition, sexual maturity of females was necessary in order to maintain this effect, since the magnitude of LTP was different between adult nulliparous female mice and sexually immature

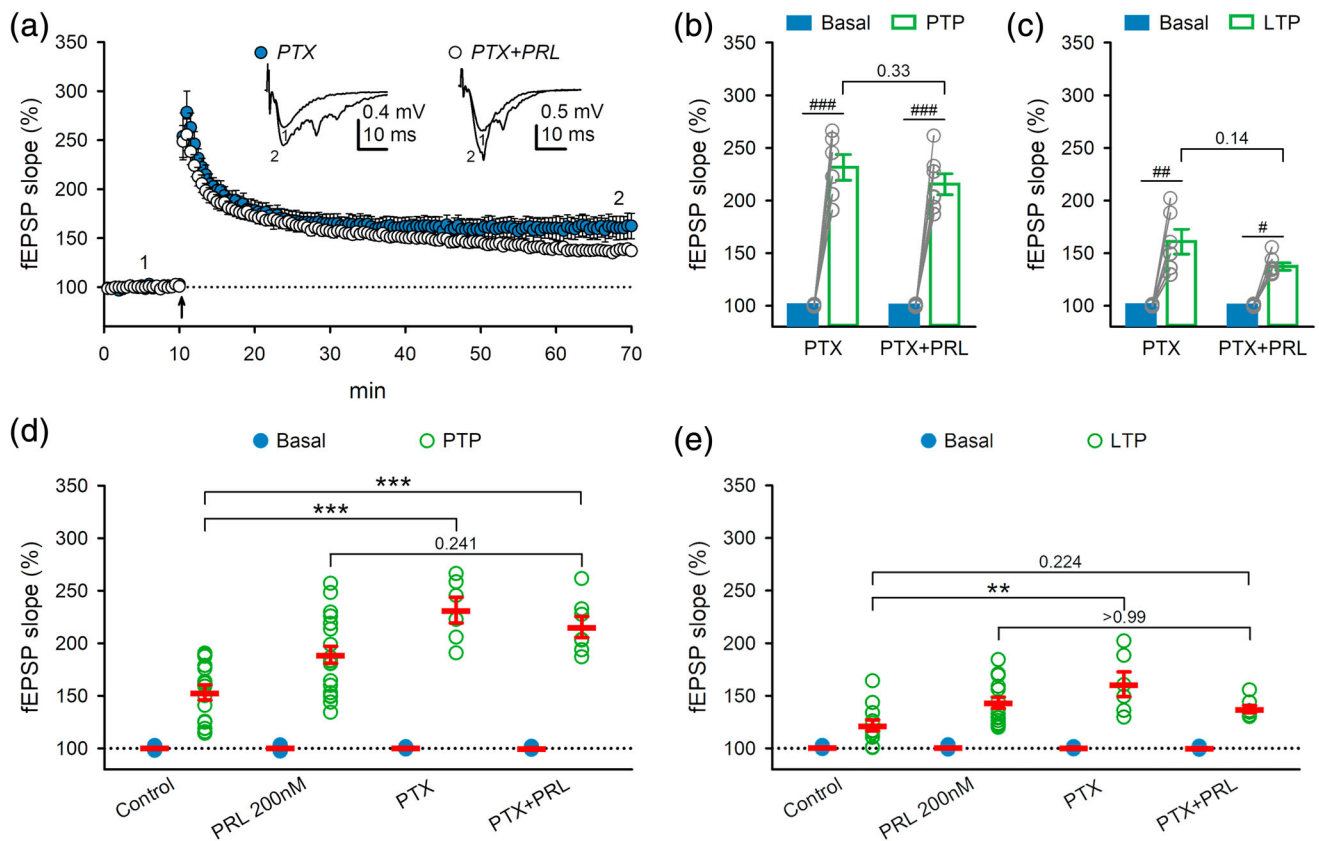


FIGURE 6 GABA_A receptors are involved in the synaptic plasticity changes induced by prolactin. (a) The enhancement of LTP induced by prolactin (PRL) was prevented by blocking GABA_A receptors with 100 μ M of picrotoxin (PTX). The insert depicts a representative superimposed recording taken during periods indicated by corresponding numbers in the graphic (1 and 2). (b) Percentage of fEPSP slope before (basal) and after TBS at 5 min (PTP) in control condition and in the presence of 200 nM PRL ($231.50 \pm 12.28\%$; $n = 6$, $N = 3$ for PTX vs. $215.53 \pm 9.94\%$, $n = 7$, $N = 3$ for PTX + PRL; $p = .3288$, $t_{[11]} = 1.022$; unpaired t test). Significant differences with respect to basal state were established with paired t test (PTX: Basal = $100.74 \pm 0.34\%$ vs. PTP = $231.49 \pm 12.28\%$; $###p < .001$, $t_{[5]} = 10.65$; $n = 6$, $N = 3$; PTX + PRL: Basal = $100.32 \pm 0.40\%$ vs. PTP = $215.53 \pm 9.93\%$, $###p < .001$, $t_{[6]} = 11.26$; $n = 7$, $N = 3$). (c) Percentage of fEPSP slope before (basal) and after TBS at 60 min (LTP) in control condition and in the presence of 200 nM PRL ($137.29 \pm 3.52\%$; $n = 7$, $N = 3$ for PTX + PRL vs. $160.94 \pm 11.81\%$; $n = 6$, $N = 3$ for PTX; Mann-Whitney test: $U_{[6,7]} = 10$, $p = .1375$). Significant differences with respect to basal state were established with paired t test to PTX ($100.74 \pm 0.34\%$; $n = 6$, $N = 3$ for Basal vs. $160.94 \pm 11.81\%$ for LTP; $n = 6$, $N = 3$; $##p = .0035$, $t_{[5]} = 5.195$) and Wilcoxon test to PTX + PRL ($100.32 \pm 0.40\%$ for Basal vs. $137.30 \pm 3.52\%$ for LTP; $n = 7$, $N = 3$; $#p = .0156$, $W = 28$). (d) Comparative plots for PTP with and without PTX (ANOVA, $F_{[3,44]} = 11.52$, $***p < .001$. Tukey's multiple comparisons test: Control vs. PTX, $***p < .001$; Control vs. PTX + PRL, $***p < .001$; PRL 200nM vs. PTX + PRL, $p = .241$ n.s.). Data taken from Figure 1c and Figure 6b. (e) Comparative plots for LTP with and without PTX (Kruskal-Wallis test, $H_{[3]} = 12.89$, $**p = .0049$. Dunn's multiple comparisons test: Control vs. PTX, $**p = .0059$; Control vs. PTX + PRL, $p = .2236$; PRL 200nM vs. PTX + PRL, $p > .99999$). Data taken from Figure 1d and Figure 6c [Color figure can be viewed at wileyonlinelibrary.com]

(P20–P27) female animals treated with PRL. In line with these results, the suppression of gonadal function modified the LTP response to acute PRL.

Different experimental approaches indicate that several cellular and molecular mechanisms are underlying PRL-dependent changes in synaptic plasticity. First, integrity of gonadal function was necessary to maintain the effect of PRL on synaptic plasticity. Second, blockage of GABA_AR with the antagonist PTX prevented the PRL effect, indicating that suppression of inhibitory activity is necessary for the changes in synaptic strength mediated by this hormone. Finally, the JAK/STAT signaling pathway was required to increase short and long-term synaptic plasticity in CA3-CA1 hippocampal synapses in the presence of PRL.

Estradiol plays a significant role in maintaining LTP and synaptic function (Fester & Rune, 2015), and estrogen from the ovaries regulates PRL secretion (Grattan, 2015). In our experimental conditions, ovariectomy occluded the PRL effect on PTP and LTP, and pre-treatment with 17 β -estradiol restored LTP magnitude to control levels. However, no significant differences were found between OVX mice treated with 17 β -estradiol alone or in the presence of PRL. This may indicate that PRL does not restore LTP levels beyond the 17 β -estradiol effect per se. The degree of rescue of LTP by estradiol in hippocampal slices from OVX rodents depends on whether this hormone is applied to the bath (Woolley, 2007) or injected systemically (Gurevicene et al., 2003). Our data showed a gradual decline of LTP in OVX mice that was not restored by systemic injection of

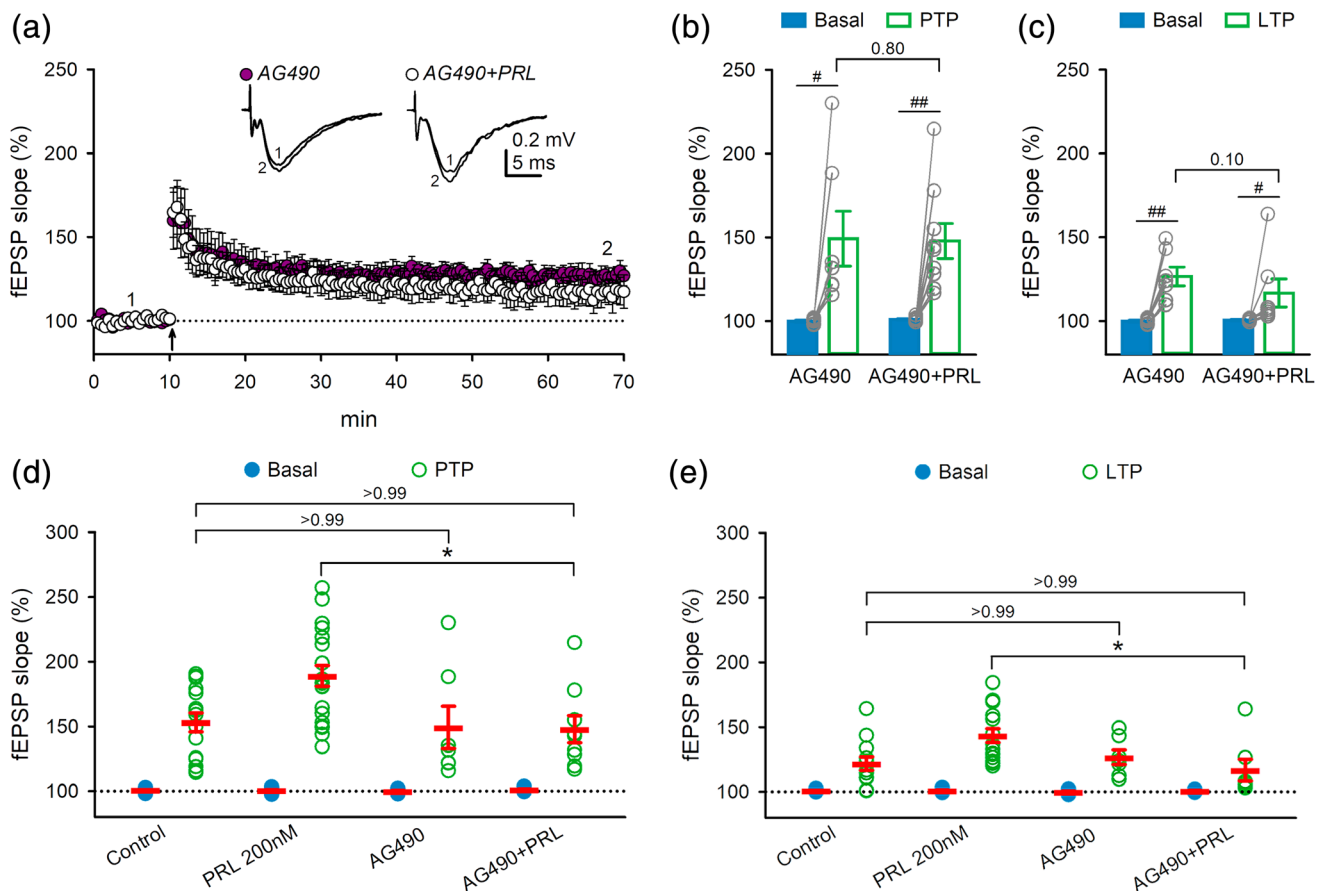


FIGURE 7 JAK/STAT signaling pathway is required for modulation of LTP by prolactin. (a) Summary data showing the time course of JAK inhibitor AG490 (10 μ M) effects on mean fEPSP slope applied alone (filled circle) or in the presence of 200 nM PRL (open circle) in basal conditions and following induction of LTP. Data were normalized for each slice with respect to the average slope recorded during baseline. Note that the JAK inhibitor AG490 prevented the enhancement of synaptic strength effect induced by PRL. The insert depicts a representative superimposed recording taken during periods indicated by corresponding numbers in the graphic (1 and 2). (b) Percentage of fEPSP slope (taken from a) before (basal) and after TBS at 5 min (PTP) in control condition and in the presence of 200 nM PRL (147.83 ± 10.47 ; $n = 9$, $N = 3$ for AG490 + PRL vs. 149.17 ± 16.32 ; $n = 7$, $N = 3$ for AG490; Mann Whitney test: $U_{[7,9]} = 29$; $p = .8054$). Significant differences in PTP with respect to basal state were established with paired Wilcoxon test for AG490 ($100.03 \pm 0.57\%$ for Basal vs. $149.17 \pm 16.32\%$ for PTP; $n = 7$, $N = 3$; $\#p = .0156$; $W = 28$) and paired t test for AG490 + PRL ($101.13 \pm 0.43\%$ for Basal vs. 147.83 ± 10.47 for PTP; $n = 9$, $N = 3$; $\#\#p = .0022$; $t_{[8]} = 4.427$). (c) Percentage of fEPSP slope before (basal) and after TBS at 60 min (LTP) in control condition and in the presence of 200 nM PRL (116.74 ± 8.41 ; $n = 7$, $N = 3$ for AG490 + PRL vs. 126.63 ± 5.59 ; $n = 7$, $N = 3$ for AG490; Mann Whitney test: $U_{[7,7]} = 11$, $p = .0973$). Significant differences in LTP with respect to basal state were established with paired t test for AG490 ($100.03 \pm 0.57\%$ for Basal vs. $126.63 \pm 5.59\%$ for LTP; $n = 7$; $N = 3$; $\#\#p = .0026$; $t_{[6]} = 4.958$) and Wilcoxon test for AG490 + PRL (100.71 ± 0.36 for Basal vs. $116.74 \pm 8.41\%$ for LTP; $n = 7$; $N = 3$; $\#p = .0156$; $W = 28$). (d) Comparative plots for PTP in the presence or absence of JAK inhibitor AG490 (Kruskal-Wallis test, $H_{[3]} = 12.11$; $\#\#p = .007$; Dunn's multiple comparisons test: Control vs. AG490, $p > .9999$; Control vs. AG490 + PRL, $p > .9999$; PRL 200nM vs. AG490 + PRL, $\ast p = .0392$). Data taken from Figure 1c and Figure 7b. (e) Comparative plots for LTP in the presence or absence of JAK inhibitor AG490 (Kruskal-Wallis test, $H_{[3]} = 12.19$, $\#\#p = .0067$; Dunn's multiple comparisons test: Control vs. AG490, $p > .9999$; Control vs. AG490 + PRL, $p > .9999$; PRL 200nM vs. AG490 + PRL, $\ast p = .0132$). Data taken from Figure 1d and Figure 7c [Color figure can be viewed at wileyonlinelibrary.com]

17 β -estradiol, in agreement with previous studies (Gureviciene et al., 2003). The interaction between hormones and their effect on synaptic plasticity is very complex. Therefore, further experiments are required in order to investigate the possible mechanisms underlying the important interaction between estradiol and PRL at the synaptic level.

It is well established that GABAergic interneurons exert a relevant influence on the occurrence of LTP by regulating the local depolarization at pre or postsynaptic levels, and relief from GABA_A inhibition

facilitates LTP induction (Bourne & Harris, 2011; Chiu et al., 2018; Grover & Yan, 1999; Wigström & Gustafsson, 1983). On the other hand, lactation alters GABA neuronal activity in the hypothalamus and cerebral cortex (Kornblatt & Grattan, 2001), raises CSF GABA concentrations (Qureshi, Hansen, & Södersten, 1987) and increases density of GABAergic synapses in the hypothalamic supraoptic nucleus (Gies & Theodosis, 1994). Furthermore, during pregnancy and after delivery, changes have been observed in hippocampal expression and function of extrasynaptic GABA_AR (Sanna et al., 2009). Considering

previous experimental evidence and our data showing that the effect of PRL on synaptic plasticity is antagonized by blockade of GABA_A receptors, it is evident that inhibition plays a fundamental role in the mechanisms responsible for the PRL enhancement of LTP.

PRL exerts its actions through the PRL_R whose activation triggers JAK2 phosphorylation (Freeman et al., 2000), which in turn phosphorylates STAT proteins (DaSilva et al., 1996). JAK2 proteins are expressed in the hippocampus and play an essential role in induction of NMDA-receptor dependent long-term depression at CA3-CA1 synapses (Nicolas et al., 2012). In addition, previous data suggest that PRL activates JAK/STAT in the hippocampus (Chiba et al., 2009; Leem, Park, Chang, Park, & Kim, 2019; Nicolas et al., 2012; Tian, Bai, Li, & Guo, 2019; Zearfoss, Alarcon, Trifilieff, Kandel, & Richter, 2008). We found that the JAK inhibitor AG490 blocks the PRL effects on induction and expression of LTP. In line with previous reports (Nicolas et al., 2012), our findings establish that JAK2/STAT pathway blockage “per se” does not modify basal synaptic transmission or the magnitude of LTP compared with control conditions. In addition, cytoplasmic activity of STAT3 plays a major role in synaptic plasticity (Nicolas et al., 2012), suggesting a postsynaptic mechanism in CA1 pyramidal neurons. Taking into account all this experimental evidence, we can hypothesize that the postsynaptic activation of JAK2/STAT3 signaling axis induced by PRL-PRL_R binding underlies the molecular mechanisms responsible for the improvement of synaptic strength induced by PRL.

In line with our findings, previous experimental evidence has demonstrated a close link between the JAK2/STAT pathway and GABA_AR. Seizure-induced decreases in GABA_AR α1 subunit expression are mediated by the JAK/STAT pathway in the hippocampus (Lund et al., 2008), and this pathway regulates GABA_AR α1 expression after traumatic cortical injury (Raible et al., 2015). Therefore, it is feasible that PRL may regulate GABAergic interneuron activity by JAK/STAT signaling. This may be a direct effect on GABAergic system, or rather is associated with a postsynaptic mechanism in CA1 pyramidal neurons (Nicolas et al., 2012) for promotion of changes in synaptic plasticity. Future studies are required to establish the specific relationship between the JAK2/STAT signaling axis, GABAergic mechanisms described in the present work and the possible localization of PRL receptor in GABAergic interneurons of the hippocampus.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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SUPPORTING INFORMATION

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