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The brain as a target of hormonal contraceptives: Evidence from animal studies

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ABSTRACT

Hormonal contraceptives are frequently prescribed drugs among women, mainly for their reversible contraceptive purposes but also for beneficial effects in some gynecological pathologies. Despite extensive studies aimed at elucidating the physical effects of hormonal contraceptives and ameliorating some unwanted outcomes, little is known yet about the effects of these drugs on brain function and related behavior, which are known to be modulated by endogenous steroid hormones. We describe the current literature on preclinical studies in animals undertaken to investigate effects of hormonal contraceptives on brain function and behavior. These studies suggest that hormonal contraceptives influence neurohormones, neurotransmitters, neuropeptides, and emotional, cognitive, social and sexual behaviors. Animals allow examination of the basic biological mechanisms of these drugs, devoid of the psychological aspect often associated to hormonal contraceptives' use in women. Understanding the neurobiological effects of these drugs may improve women's health and may help women making informed choices on hormonal contraception.

1. Introduction

Since their introduction in 1960 as a reliable approach to birth control, hormonal contraceptives are currently some of the most prescribed drugs in the world. The "pill" is a combination of synthetic estrogens and progestins (combined oral contraceptives). Ethinyl estradiol (EE) is the most used estrogen component, although estradiol valerate or estetrol have been found to exert the same contraceptive action with less untoward effects than EE (Apter et al., 2017; Nappi et al., 2014; Sech and Mishell, 2015). Estrogens can be paired to several classes of progestins, including the androgenic nortestosterone derivatives levonorgestrel (LNG), norethindrone, desogestrel, gestodene, norgestimate, the anti-androgenic progesterone derivatives cyproterone acetate, chlormadinone acetate, nomegestrol acetate, and the spironolactone derivative drospirenone (Sech and Mishell, 2015; Sitruk-Ware and Nath, 2010). Progestin only pills are also available and the current generation, which has similar contraceptive efficacy as combined preparations, is preferred for the limited vascular effects (Regidor, 2018). Emergency contraception also consists of a single administration of progestins at a higher dose, usually LNG 1.5 mg or ulipristal acetate 30 mg (Regidor, 2018). In addition to the pill, a variety of contraceptive formulations are also available, including injections, skin patches, subdermal implants, vaginal rings, and intrauterine devices that release hormones (Levin et al., 2018).

The success of these agents has resulted from the high efficacy, rapid reversibility of their effects, and extra non-contraceptive medical purposes, including treatment of polycystic ovary syndrome, endometriosis, dysmenorrhea, acne and hirsutism. Hormonal contraceptives can also help to prevent and treat iron-deficiency anemia and offer a protective effect against endometrial and ovarian cancer. No major health risks have been reported; however, some concerns include an increase in relative risk for venous thromboembolism in women who smoke or have other predisposing factors to thrombosis or thromboembolism, and risk for breast cancer. In addition, their use in some women is associated with breakthrough menstrual bleeding, nausea, headache, edema, and decreased sexual desire (De Leo et al., 2016; Levin et al., 2018; Powell, 2017).

The majority of women who take the oral contraceptive pill or use other forms of hormonal contraception reports no change or an improvement in psychological well-being (Bitzer et al., 2018; Bottcher et al., 2012; Keyes et al., 2013; McKetta and Keyes, 2019; Rapkin et al., 2006b; Schaffir et al., 2016; Toffol et al., 2012); however, irritability and episodes of affective disorders, such as depression or mood changes, remain a problem for approximately 4–10% of hormonal contraceptives' users (Lundin et al., 2017; Segebladh et al., 2009; Skovlund et al., 2016; Sundstrom Poromaa and Segebladh, 2012), and

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are among the most common reasons given by women for discontinuing their effective use, often within the first three months, suggesting psychological effects of these drugs (Goldzieher, 1994; Hall et al., 2012; Kulkarni, 2007; Kurshan and Epperson, 2006; Rapkin et al., 2006a; Sanders et al., 2001). In addition, hormonal contraceptives' users show altered volumes and microstructural changes in specific brain regions, as well as decreased functional connectivity in the frontal nodes of the executive network, implicating important consequences for regulation of brain function (De Bondt et al., 2013; Petersen et al., 2014; Pletzer et al., 2016, 2010).

All together, the above-mentioned studies clearly demonstrate that hormonal contraceptives affect brain function, and that their effects at the level of the central nervous system (CNS) may be a problem for a significant group of contraceptives' users. However, although alterations in cognitive domains and affective states induced by hormonal contraceptives have recently gained particular attention (Montoya and Bos, 2017; Pletzer and Kerschbaum, 2014), the mechanisms underlying their effects on brain function have been poorly examined. Here, we describe the current literature on preclinical studies in animals, undertaken to investigate the effects of hormonal contraceptives on the CNS. Animal models represent a valuable approach to investigate the mechanisms by which drugs exert their effects in the brain; nonetheless very few studies have focused on the effects of hormonal contraceptives in animals as a means to investigate their action on the brain. It must be acknowledged that most of the studies in animals have used the androgenic LNG as a progestin component of the contraceptive formulation, and results may not generalize to all progestins currently available. An extensive comparative study on the effect of the different progestin compounds and possibly on the estrogen ratios of combined formulations would be important to elucidate the actions of hormonal contraceptives on brain and behavior. Nonetheless, animals represent a useful experimental model to merely examine the basic biological mechanisms of hormonal contraceptives, devoid of the psychological aspect often associated to contraceptive use in women.

2. Effects of hormonal contraceptives on levels of steroid hormones

The synthetic steroids used as hormonal contraceptives, by binding to the estrogen and progesterone receptors, disrupt the hypothalamic-pituitary-gonadal (HPG) axis. In fact, hormonal contraceptives prevent ovulation by inhibiting the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary, and thus, gonadal steroids from the ovary; hence, hormonal contraceptives prevent the increase in circulating levels of estrogen and progesterone typically observed throughout the menstrual cycle (Lobo and Stanczyk, 1994; Paoletti et al., 2004; Rapkin et al., 2006b). Moreover, hormonal contraceptives prevent the increase in circulating levels of pregnenolone, and of the progesterone derivative, allopregnanolone ((3α,5α)-3-hydroxypregnan-20-one or 3α,5α-THP), which typically occurs in the luteal phase of the menstrual cycle (Follesa et al., 2002; Paoletti et al., 2004; Rapkin et al., 2006a, 2006b) (summarized in Fig. 1). Allopregnanolone is a neuroactive steroid involved in several neuropsychiatric disorders, including anxiety, depression, premenstrual dysphoric disorder, post-traumatic stress disorder, or alcohol dependence (Backstrom et al., 2014; Girdler et al., 2012; Porcu et al., 2016; Porcu and Morrow, 2014; Rasmusson et al., 2017; Wang et al., 1996).

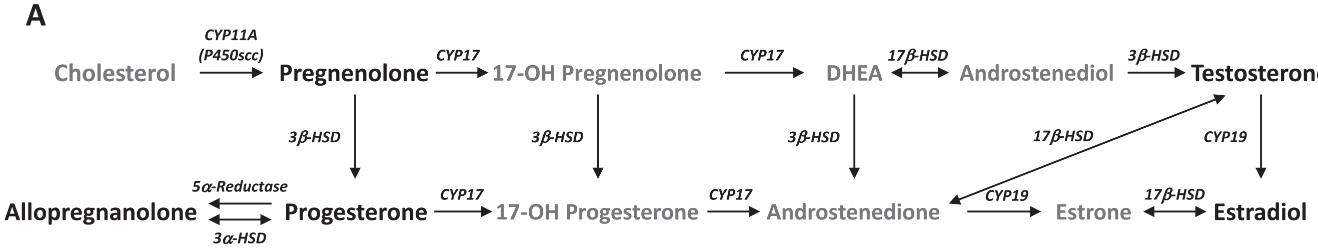
Likewise, hormonal contraceptives disrupt the estrus cycle in rats, and inhibit ovulation with a subsequent decrease in HPG axis function and neuroactive steroid levels. Chronic administration (up to 4 weeks, a time that covers approximately 6–7 estrus cycles) of EE and LNG, alone or in combination, as well as of EE in combination with other progestins (desogestrel, norethisterone, chlormadinone acetate or cyproterone acetate) markedly reduced basal and GnRH-stimulated serum LH levels in intact female rats (Kuhl et al., 1984). GnRH-stimulated, but not basal LH levels normalized after a 2-week discontinuation period (Kuhl et al., 1984). In agreement with these findings, we showed that chronic administration (up to 6 weeks, a time that covers approximately 8–10 estrus cycles) of EE (30 µg), LNG (125 µg), and their combination (EE-LNG) markedly reduced plasma, cerebrocortical and hippocampal levels of neuroactive steroid hormones in adult female Sprague-Dawley rats, compared to vehicle-treated rats randomly assessed throughout the estrus cycle. Specifically, progesterone levels were reduced by ~70% in the cerebral cortex, ~58% in the hippocampus and ~46% in plasma. Levels of its precursor pregnenolone were decreased by ~40% in the cerebral cortex, ~54% in the hippocampus and ~38% in plasma. Levels of their neuroactive metabolite allopregnanolone were reduced by ~72% in the cerebral cortex, ~62% in the hippocampus and ~41% in plasma (Follesa et al., 2002; Porcu et al., 2012; Sassoè-Pognetto et al., 2007). Alterations in levels of these neuroactive hormones normalized two weeks after treatment discontinuation (Follesa et al., 2002). Long-term administration of EE-LNG also decreased cerebrocortical concentrations of testosterone (−77%), compared to vehicle-treated rats randomly assessed throughout the estrus cycle (Santoru et al., 2014), an effect similar to that reported in women using hormonal contraceptives, who also have lower serum testosterone levels (Graham et al., 2007; Paoletti et al., 2004). These findings are summarized in a diagram in Fig. 1 along with a simplified schematic of the biosynthetic pathway for the above-mentioned neuroactive steroids.

Hormonal contraceptives also alter estradiol concentrations in female Sprague-Dawley rats. Administration of LNG (125 µg) for five days is sufficient to decrease serum estradiol levels and to alter the estrus cycle, compared to untreated rats in proestrus (naturally high estradiol), but not in metestrus (naturally low estradiol) (Graham and Milad, 2013). Interestingly, estradiol levels normalized within five days from LNG suspension, or following treatment with agonists for both types of estrogen receptors (ER) ERα and ERβ, which, within an hour, increased estradiol levels to values comparable to those in proestrus rats (Graham and Milad, 2013). Another study reported that a three-weeks administration of EE alone (30 µg), LNG alone (60 µg), or their combination decreased serum estradiol levels, with respect to vehicle-treated animals in the early diestrus/metestrus phase of the ovarian cycle, when estradiol levels are naturally low compared to late diestrus and proestrus phases. By contrast, lower doses of EE (10 µg), LNG (20 µg), or their combination did not alter estradiol levels (Simone et al., 2015), suggesting that higher doses of EE and LNG may be required to inhibit the HPG axis in rodents.

The molecular mechanisms by which hormonal contraceptives decrease neuroactive steroid levels are not completely understood. They may depend on inhibition of the HPG axis, but may also include effects of EE and/or LNG on enzymatic expression and activity. In fact, in cultured rat luteal cells LNG inhibits basal and LH-stimulated progesterone synthesis, as well as the conversion of pregnenolone to progesterone, suggesting a possible direct action on 3β-hydroxysteroid dehydrogenase (Telleria et al., 1994). Likewise, LNG inhibits the activity of 5α-reductase in skin (Rabe et al., 2000). The effects of hormonal contraceptives on expression and activity of neurosteroidogenic enzymes in the brain are still unknown. Nonetheless, our observation that the decrease in cerebral cortical concentrations of pregnenolone, progesterone and allopregnanolone induced by long-term EE-LNG treatment is still present in ovariectomized rats (Follesa et al., 2002), suggests that hormonal contraceptives may exert a direct effect on brain neurosteroidogenesis, independent of the ovarian source.

Given that estradiol, progesterone and allopregnanolone exert numerous beneficial effects including mood enhancing effects, neuroprotection, and modulation of neuronal plasticity and cognition (Guennoun et al., 2015; Luine, 2014; Porcu et al., 2016), the decrease in their concentrations induced by hormonal contraceptives may be deleterious to the normal brain function and may contribute to some of the effects that these drugs exert at the CNS level.

A



B

Neuroactive Steroids	Female Rats			Women
	Cerebral Cortex	Hippocampus	Plasma	Plasma
Pregnenolone	↓ (1,5,8)	↓ (5,9)	↓ (1,5)	↓ (1,6,7)
Progesterone	↓ (1,5,8)	↓ (5,9)	↓ (1,5)	↓ (1,4,6,7)
Allopregnanolone	↓ (1,5,8)	↓ (5,9)	↓ (1,5)	↓ (1,4,6,7)
Estradiol			↓ (2,10)	↓ (4,6,7)
Testosterone	↓ (8)			↓ (3,4)

Fig. 1. Biosynthetic pathway for selected neuroactive steroids (A) and summary of changes in their concentrations induced by long-term treatment with hormonal contraceptives in female rats and women (B). (A) Abbreviations: DHEA, dehydroepiandrosterone; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 3α-HSD, 3α-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase. (B) The arrow facing down indicates a decrease in neuroactive steroid levels. Numbers in brackets are the following references: 1. Follesa et al. (2002); 2. Graham and Milad (2013); 3. Graham et al. (2007); 4. Paoletti et al. (2004); 5. Porcu et al. (2012); 6. Rapkin et al. (2006a); 7. Rapkin et al. (2006b); 8. Santoru et al. (2014); 9. Sassoè-Pognetto et al. (2007); 10. Simone et al. (2015).

3. Effects of hormonal contraceptives on neurotransmission

There is weak evidence in animals regarding the effect of hormonal contraceptives on neurotransmission, in spite of the fact that these exogenous steroid hormones disrupt HPG axis function, whose regulation is suggested to depend on several neurotransmitters, such as monoamines, acetylcholine, glutamate and GABA.

3.1. Monoamines and acetylcholine

Chronic treatment (for 4 or 30 days) with EE in combination with different progestins used in hormonal contraceptives' preparations has been shown to increase brain levels of serotonin and its aminoacid precursor tryptophan in rats, while reducing striatal concentrations of dopamine in mice and rats (Baker et al., 1977; Daabees et al., 1981; Jori and Dolfini, 1976). Moreover, estradiol, alone or in combination with norgestrel, was reported to inhibit monoamine oxidase activity (Marchi and Cugurra, 1974) and the combination of EE-LNG at low doses (10–20 µg) induced a decrease in tyrosine hydroxylase mRNA and protein in the locus coeruleus, while higher doses (30–60 µg) increased tyrosine hydroxylase mRNA but not protein levels (Simone et al., 2015). EE alone inhibits, with comparable efficacy, both the serotonergic and the catecholaminergic transporters (Ghraf et al., 1983; Michel et al., 1987). In contrast, hormonal contraceptives (chronic 12/30-day treatment) did not alter acetylcholine and choline brain levels, and choline acetyltransferase activity in both mice and rats (Daabees et al., 1981; Ladinsky et al., 1976). The majority of these studies were based on older contraceptive formulations and the findings may not generalize to the current contraceptives. More studies are required to test the effects of the current formulations of hormonal contraceptives on neurotransmission.

3.2. Aminoacids

Hormonal contraceptives increase γ-aminobutyric acid (GABA) levels in the rat brain, without affecting glutamate levels (Daabees et al., 1981). Moreover, we demonstrated that hormonal contraceptives alter $GABA_A$ receptor subunits expression in different areas of the rat brain (Follesa et al., 2002; Porcu et al., 2012). $GABA_A$ receptors are heteromeric complexes of five transmembrane subunits assembled to form a channel permeable to chloride ions. The most abundant receptors are typically formed by two α, two β, and a γ subunit, which is replaced by the δ subunit in the extrasynaptic receptors. In addition to the binding site for GABA, $GABA_A$ receptors are the target for several drugs, including benzodiazepines, barbiturates, general anesthetics, alcohol, as well as for the endogenous 3α,5α/3α,5β-reduced neuroactive steroids, including allopregnanolone (Sieghart and Savic, 2018).

Long-term treatment (4–6 weeks) with the EE-LNG combination (30–125 µg/rat, respectively) increased cerebral cortical mRNA expression for the γ2L and γ2S isoforms (+33% and +22%, respectively), compared to vehicle-treated controls randomly assessed throughout the estrus cycle; such changes were still evident one week after discontinuation, and normalized by two weeks from drug discontinuation (Follesa et al., 2002). The abundance of the γ2 protein was increased by 70% in the cerebral cortex (Follesa et al., 2002) and by 43% in the hippocampus (Porcu et al., 2012) of EE-LNG-treated rats, compared to vehicle-treated rats in random phases of the estrus cycle. By contrast, long-term EE-LNG treatment did not alter α1, α3, α4, α5, β1, β2, and β3 subunits mRNA expression and relative proteins in both cerebral cortex and hippocampus (Follesa et al., 2002; Porcu et al., 2012), indicating that changes in the abundance of the γ2 subunit mRNAs and protein are specific. However, the functional relevance of such alterations in subunit expression remains to be clarified.

Interestingly, EE-LNG treatment also increased γ2 subunit expression in ovariectomized rats, suggesting that this effect is not influenced by peripheral steroid hormones whose plasma concentrations were not affected by such treatment in rats in which gonads have been removed (Follesa et al., 2002). Rather, the effect of EE-LNG on $GABA_A$ receptor subunit expression may be related to the decrease in brain allopregnanolone levels induced by these drugs (Follesa et al., 2002; Porcu et al., 2012; Sassoè-Pognetto et al., 2007). Allopregnanolone is a potent positive endogenous modulator of $GABA_A$ receptors, through which it exerts several psychopharmacologic actions, such as anxiolytic, antidepressant, anticonvulsant, sedative, anesthetic, analgesic and amnesic effects (Carver and Reddy, 2013; Porcu et al., 2016). Moreover, changes in allopregnanolone levels that occur in physiological conditions such as puberty (Shen et al., 2007), the estrus cycle (Lovick et al., 2005; Maguire et al., 2005), pregnancy and lactation (Concas et al., 1998; Maguire and Mody, 2008; Mostallino et al., 2009; Sanna et al., 2009), or following pharmacological manipulations (Calza et al., 2010; Follesa et al., 2001; Locci et al., 2017; Modol et al., 2014; Shen et al., 2005; Smith et al., 1998) influence $GABA_A$ receptor subunit expression.

On the other hand, the alterations in $GABA_A$ receptor subunit expression induced by long-term EE-LNG treatment may not be strictly related to the concomitant decrease in brain allopregnanolone levels. In fact, long-term treatment with LNG alone, but not with EE alone, alters $GABA_A$ receptor subunit expression, despite both treatments markedly decreased allopregnanolone levels and its precursors in brain and plasma. Specifically, long-term treatment with LNG alone, similar to the EE-LNG combination, induced an increase in the abundance of the γ2 subunit in the cerebral cortex and the hippocampus (+38% and +59%, respectively), while long-term treatment with EE alone was ineffective. Likewise, long-term treatment with LNG alone also decreased the abundance of the α1 subunit in the cerebral cortex (-21%), but not in the hippocampus; further cerebrocortical and hippocampal levels of this subunit were also not altered by long-term treatment with EE alone, or with the EE-LNG combination (Porcu et al., 2012). Therefore, such changes in subunit expression are not strictly related to the EE- and LNG-induced decrease in brain allopregnanolone levels, but might be directly mediated by LNG.

The molecular mechanisms through which LNG may induce such effects remain unclear. Although LNG exhibits a potent progesterone-like activity, it lacks estrogen-like effects (Kuhl, 1996), but it also binds with high affinity to androgen receptors (Cabeza et al., 1995; Lemus et al., 1992) and to intracellular mineralocorticoid receptors (Kuhl, 1996). Furthermore, LNG is metabolized into A-ring reduced derivatives, including 3α,5α- and 3α,5β-LNG, which have enhanced binding affinity for androgenic receptors compared to LNG (Lemus et al., 1992). Thus, the possibility that LNG or its metabolites might alter γ2 and α1 subunit expression via androgen receptors cannot be ruled out; in fact chronic exposure to androgenic steroids also regulates $GABA_A$ receptor expression (Henderson et al., 2006; McIntyre et al., 2002). Nonetheless, given that $GABA_A$ receptors are a target for neuroactive steroids (Carver and Reddy, 2013), it is also possible that LNG might affect $GABA_A$ receptor subunit expression through its 3α,5α-and 3α,5β-reduced metabolites directly interacting with this receptor system, although such direct action has not been demonstrated to date. Given the importance of $GABA_A$ receptor-mediated neurotransmission in the modulation of brain function and its involvement in the regulation of a variety of psycho-physiologic phenomena, including anxiety, depression, sleep, cognitive function, seizures and sexual behavior, besides the exact mechanisms through which LNG or the EE-LNG combination affect $GABA_A$ receptor subunit expression, the changes in $GABA_A$ receptors induced by EE-LNG treatment might be relevant to some of the effects sometimes exhibited by women using hormonal contraceptives.

3.3. Neuropeptides

Brain-derived neurotrophic factor (BDNF) has been implicated in the regulation of mood disorders and it is also thought to mediate cognitive functions through a role in long-term potentiation, a form of synaptic plasticity that is thought to underlie the formation of long-term memory (Bekinschtein et al., 2014). Estradiol enhances the expression of BDNF mRNA and protein in hippocampus and amygdala (Zhou et al., 2005), through a direct regulation of an estrogen response element identified in the BDNF gene (Sohrabji et al., 1995). Moreover, progesterone and allopregnanolone also increase the expression of this neurotrophin in different brain regions (Almeida et al., 2019; Naert et al., 2007). Accordingly, BDNF expression in the hippocampus fluctuates with estrus cycling in rats, in relation to the changes in steroid levels (Scharfman et al., 2003). Long-term treatment with EE and LNG significantly reduces BDNF mRNA and protein in the hippocampus of female rats (Simone et al., 2015), an effect most likely related to the decrease in brain and plasma levels of steroid hormones induced by such treatment. Similarly, serum BDNF levels fluctuate in women over the menstrual cycle with a peak occurring during the luteal phase; in contrast, no luteal phase augmentation in serum BDNF occurs in women taking hormonal contraceptives (Pluchino et al., 2009).

Finally, EE and LNG alter the expression of galanin, a stress-inducible neuropeptide and cotransmitter in serotonergic and noradrenergic neurons, with a possible role in stress-related disorders (Juhasz et al., 2014). Specifically, low dose EE (10 μg), alone or in combination with LNG, increases galanin peptide in the locus coeruleus, while higher EE doses (30 μg), despite increasing galanin mRNA, do not alter protein content (Simone et al., 2015). Galanin may inhibit noradrenergic transmission, a mechanism that has been hypothesized to be involved in the EE-induced changes in anxiety and cognition (Simone et al., 2015).

4. Behavioral effects of hormonal contraceptives

4.1. Anxiety and depression

Women are more likely than men to develop affective disorders, including depression and anxiety, and hormonal fluctuations associated with reproductive function may account for this increased vulnerability to mood disorders, beginning with a heightened risk of developing a depressive episode following puberty (Barth et al., 2015; Hantsoo and Epperson, 2017). Hormonal contraceptives have been reported to affect mental health in women, although the results are often contradictory. In fact, amelioration (Freeman et al., 2012; Herzberg et al., 1971; Toffol et al., 2012), no change (Rapkin et al., 2006b), or an increase (Deci et al., 1992; Oinonen and Mazmanian, 2002; Skovlund et al., 2016) in symptoms of depression and/or anxiety have been reported in several studies among women using the oral contraceptive pill, as well as other forms of hormonal contraception. A genetic predisposition to mood disorders may account for these discrepancies; in fact, depression and mood changes approximately affect 4–10% of hormonal contraceptives' users, who may be predisposed to more likely experience these effects of the "pill" (Hamstra et al., 2017, 2016, 2015; Rapkin et al., 2006b; Schaffir et al., 2016; Segebladh et al., 2009; Sundstrom Poromaa and Segebladh, 2012). Accordingly, depressogenic effects of hormonal contraceptives were observed at a significant higher level in carriers of mineralocorticoid receptor haplotype 1 or 3, while carriers of the haplotype 2 seem to be protected against these negative effects on mood during their reproductive years (Hamstra et al., 2015).

Recently, two huge prospective cohort studies strongly demonstrated that the use of different types of hormonal contraception, especially among adolescents, was associated with subsequent use of psychotropic drugs and a first diagnosis of depression, supporting depression as a potential untoward effect of these agents (Skovlund et al., 2016; Zettermark et al., 2018). Use of hormonal contraceptives was also positively associated with subsequent suicide attempt and suicide, with adolescents experiencing the highest relative risk (Skovlund et al., 2018). Adolescent girls show higher rates of depression and anxiety than boys, and steroid hormones might account for this discrepancy, given their role in shaping brain development during the transition from childhood to adulthood (Juraska et al., 2013; Juraska and Willing, 2017; Naninck et al., 2011). Thus, given that adolescents use hormonal contraceptives for medical and contraceptive purposes (Abma and Martinez, 2017), much attention should be paid to their effects on brain function. In fact, intake of synthetic estrogens and progestins, in addition to preventing the normal HPG axis function, may affect brain development in adolescents in ways that have not yet been explored (Cahill, 2018).

Endogenous steroid hormones modulate emotional behavior in animal models (Borrow and Handa, 2017; Porcu et al., 2016). We thus hypothesized that the decrease in the concentrations of estradiol, progesterone and its neuroactive metabolite allopregnanolone, induced by long-term administration of hormonal contraceptives would affect anxiety- and depressive-like behavior in female rats. Accordingly, allopregnanolone concentrations are decreased in plasma and cerebrospinal fluid of depressed patients and can be normalized by antidepressant treatment (Uzunova et al., 1998), strongly suggesting that allopregnanolone may be involved in the etiology of depression (Backstrom et al., 2014).

4.1.1. Anxiety-like behavior

We reported that long-term administration of EE-LNG (30–125 μg/rat, respectively) induced an anxiety-like behavior in the elevated plus maze test (Follesa et al., 2002). This effect was likely mediated by LNG or its metabolites, since long-term administration of EE alone, which also decreases progesterone and allopregnanolone levels, did not alter anxiety-like behavior, while LNG alone increased such behavior (Porcu et al., 2012). LNG binds to progesterone receptors, as well as to androgen receptors through which it exerts a potent androgenic activity. In addition, it can be A-ring reduced to mainly form 5α-LNG and 3α,5α-LNG, and to a lesser extent 3β,5α-LNG (Lemus et al., 1992), which may have biological relevance for CNS effects. In fact, acute administration of 3β,5α-LNG and 3α,5α-norethisterone at the higher doses induced an anxiolytic effect in ovariectomized Wistar rats, while acute administration of LNG or norethisterone did not alter anxiety-like behavior in the burying behavior test (Picazo et al., 1998). Likewise, a long-term exposure to LNG induced anxiety, mood disorders, and sleep problems in otherwise healthy women without a prior record of these events (Slattery et al., 2018; Wagner and Berenson, 1994).

A recent study reported that chronic administration of low doses of EE and LNG (10 and 20 μg, respectively), which do not alter estradiol, progesterone and allopregnanolone levels (Concas et al. unpublished observation), were found to exert an anxiolytic effect in the elevated plus maze test and the shock-probe defensive burying test (Simone et al., 2015). By contrast, higher doses of combined EE and LNG (30 and 60 μg, respectively) did not alter anxiety-like behavior in the elevated plus maze test, although EE alone (30 μg) increased freezing time in the shock-probe defensive burying test (Simone et al., 2015). Moreover, no effects of EE and LNG on object bias and locomotor activity/exploration in the open field were reported (Simone et al., 2015). Thus, hormonal contraceptives may modulate anxiety-like behavior through multiple different mechanisms; future studies in rodents are necessary to elucidate these mechanisms in order to better target potential effects of these drugs on anxiety states.

4.1.2. Depression-like behaviors

We examined the effect of long-term treatment with EE-LNG (30–125 μg/rat, respectively) on depressive-like behavior in the sucrose preference test, which measures anhedonia, a core symptom of depression (Willner, 1997), and in the forced swim test, which evaluates the antidepressant activity of a drug (Porsolt et al., 1977). In contrast to our hypothesis that a decrease in allopregnanolone concentrations induced by long-term EE-LNG treatment might induce a depressive-like behavior, we found that such treatment did not alter sucrose intake in female rats (Santoru et al., 2014). In the forced swim test we found that, on test day, immobility time was decreased in EE-LNG-treated rats compared to vehicle-treated rats (Santoru et al., 2014), which, according to the original interpretation by Porsolt (Porsolt et al., 1977), suggests an antidepressant effect of hormonal contraceptives. However, interpretation of the antidepressant effect of a drug in the forced swim test has recently been challenged, since this test may not be suitable to detect antidepressant-like behavior in the presence of stress and anxiety (Estanislau et al., 2011; Molendijk and de Kloet, 2015). In fact, it has been proposed that immobility in the forced swim test may represent a switch from active to passive behavior in order to cope with an acute stressor (Molendijk and de Kloet, 2015). In line with this interpretation, the decreased immobility observed in EE-LNG-treated rats might underlie an inability of these rats to cope with the acute swim stress, rather than a change in depressive-like behavior. Indeed, hormonal contraceptives have been found to blunt the hypothalamic-pituitary-adrenal (HPA) axis response to acute stress in women (Kirschbaum et al., 1999, 1995), suggesting that they may alter sensitivity to stress.

4.1.3. Response to stress

Alterations in HPA axis function in response to stress increase vulnerability to development of mood disorders, and HPA axis hyperactivity has been frequently reported in depressed patients (Stetler and Miller, 2011). Interestingly, studies in women have shown that hormonal contraceptives alter basal and stress-stimulated HPA axis responses. Specifically, hormonal contraceptives increase basal circulating cortisol (Kirschbaum et al., 1999; Paoletti et al., 2004; Wiegratz et al., 2003), although effects on free salivary and plasma cortisol are divergent and inconclusive (Boisseau et al., 2013; Kirschbaum et al., 1999; Reynolds et al., 2013), and may depend on genotype (Hamstra et al., 2015). Further, hormonal contraceptives blunt the free cortisol response to both psychosocial stress and pharmacological activation of the HPA axis with naltrexone, or adrenocorticotropic hormone (ACTH) (Kirschbaum et al., 1999, 1995; Roche et al., 2013). Likewise, a corticotropin releasing hormone (CRH) challenge blunted plasma ACTH levels in hormonal contraceptives' users (Jacobs et al., 1989). The hormonal contraceptives-induced changes in HPA axis function may depend upon a direct action of EE on corticosteroid binding globulin (CBG). In fact, EE dose-dependently increased CBG levels, while the progestin component was ineffective (Wiegratz et al., 2003). Moreover, hormonal contraceptives' users with CBG levels higher than the median value also showed blunted ACTH and salivary cortisol responses to psychosocial stress, while the total cortisol response was increased, compared to those users with CBG levels below the median value (Kumsta et al., 2007). Thus, the increase in CBG content induced by EE may account for the blunted HPA response to stress in hormonal contraceptives' users. In line with this interpretation it has been reported that the use of a LNG-releasing intrauterine device was associated with an increased salivary cortisol response to stress (Aleknaviciute et al., 2017), suggesting that the effects of hormonal contraceptives on the response to stress may depend upon the formulation used and may involve different mechanisms.

We tested the effects of long-term treatment with EE-LNG (20–60 μg/rat, respectively) on basal and stress-induced corticosterone levels in female Sprague-Dawley rats. EE-LNG increased (+43%) basal plasma corticosterone levels, compared to vehicle-treated rats

Table 1
Long-term treatment with EE-LNG blunts the corticosterone and allopregnanolone responses to acute stress.

	Vehicle	EE-LNG
Corticosterone		
No stress	56.0 ± 15.3	79.9 ± 20.8[a]
Acute stress	541.4 ± 36.8[d]	407.6 ± 29.6[a,d]
Allopregnanolone		
No stress	17.6 ± 1.9	10.1 ± 0.4[b]
Acute stress	23.8 ± 1.9[c]	12.0 ± 0.7[b]

60 days old Sprague-Dawley female rats were orally treated with the combination of EE and LNG (20–60 μg/rat, respectively), once daily for 4 weeks. Control rats received the same amount of vehicle (1 ml, 0.4% sodium carboxymethylcellulose), and were randomly assessed throughout the estrus cycle. 24 h after the last administration, rats were subjected to an acute 30 min restraint stress, and sacrificed thereafter for measurement of plasma hormone levels. Rats in the no stress group were left undisturbed in their home cage. Plasma corticosterone and allopregnanolone levels (ng/ml) were measured by radioimmunoassay as described (Porcu et al., 2017). Data are means ± SEM of values from 8 rats per group. [a]$p < 0.05$, and [b]$p < 0.01$ vs. the respective vehicle-treated group; [c]$p < 0.05$, and [d]$p < 0.0001$ vs. the respective no stress group; two-way ANOVA, followed by Bonferroni post-hoc test.

randomly assessed throughout the estrus cycle (Table 1). The decrease in basal allopregnanolone levels induced by long-term EE-LNG treatment (Follesa et al., 2002; Porcu et al., 2012; Santoru et al., 2014) may contribute to the elevation in basal corticosterone levels, given that allopregnanolone, by acting upon hypothalamic GABAergic neurons, regulates basal HPA homeostasis (Biggio et al., 2014; Gunn et al., 2015). Furthermore, long-term EE-LNG treatment blunted the corticosterone response to acute restraint stress. Specifically, acute stress increased corticosterone levels by 866% in vehicle-treated rats, and by 410% in EE-LNG-treated rats, compared to the respective non-stressed rats; thus hormonal contraceptives elicit a two-fold reduction in the corticosterone response to stress, compared to vehicle treatment (Table 1), indicative of a blunted HPA axis activity and reduced sensitivity to stress. These results appear in agreement with the clinical findings in women, although future studies are required to further examine the mechanisms by which hormonal contraceptives affect the response to stress in female rats, including evaluation of CBG content, as well as of total and free corticosterone levels.

By contrast, allopregnanolone may not be involved in the regulation of the response to stress in EE-LNG-treated rats. In fact, we found that, compared to the respective non-stressed rats, acute stress increased allopregnanolone levels by 35% in vehicle-treated rats, but did not alter its levels in EE-LNG-treated rats (Table 1), suggesting that EE-LNG-treated rats are lacking the allopregnanolone response to stress, necessary to restore the stress-induced changes in HPA axis homeostasis (Biggio et al., 2007; Gunn et al., 2015).

In summary, long-term EE-LNG treatment blunts the corticosterone and allopregnanolone responses to acute stress, suggesting that EE-LNG-treated rats may be more vulnerable to the effects of stress, a factor that may predispose to increased susceptibility to affective disorders.

4.1.4. *Fear extinction*

A useful experimental paradigm to study regulation of emotions is fear conditioning and extinction, a procedure that also allows to study the mechanisms underlying emotional learning. Hormonal contraceptives impair fear extinction in healthy women; in fact, women taking combined oral contraceptives showed higher recovery of fear compared to free-cycling women during the high estrogen phase, suggesting that fear responses persist for a longer period in hormonal contraceptives' users. No alterations in conditioning or acquisition of extinction were reported (Graham and Milad, 2013). Likewise, LNG dose-dependently impaired extinction recall in female rats, without altering acquisition of fear and extinction (Graham and Milad, 2013). However, a deficit in extinction recall was only observed in those rats that were on LNG treatment during extinction training, while rats that terminated LNG treatment 4 days before extinction training did not show any impairment in extinction recall. These effects of LNG may depend upon the reduction in estradiol levels induced by such treatment. In agreement, administration of an ERα or ERβ agonist to LNG-treated rats decreased freezing during extinction recall, suggesting that the impairment in extinction recall may be due to the low estradiol available to bind ERs. Likewise, estradiol administration to naturally cycling women prevented recovery of fear during extinction recall, suggesting that this hormone enhances the consolidation of extinction (Graham and Milad, 2013). However, the possibility that hormonal contraceptives-induced changes in progesterone and allopregnanolone may contribute to the impaired extinction recall cannot be excluded, given the contribution of these hormones to learning and memory as well as to fear extinction (Milad et al., 2009; Pibiri et al., 2008).

4.2. *Cognition*

Clinical studies suggest that gonadal hormones modulate cognitive processes. The outcomes on the use of hormonal contraceptives on women's performance on cognitive tasks are numerous, but still inconclusive, probably due to the different cognitive domains analyzed (verbal and spatial abilities, executive function, attention, etc.) and to the type of progestin administered (Gogos et al., 2014; Pletzer and Kerschbaum, 2014; Warren et al., 2014). In particular, an improvement (Rosenberg and Park, 2002), no difference (Islam et al., 2008; Mihalik et al., 2009; Mordecai et al., 2008), or a worsening (Griksiene et al., 2018; Griksiene and Ruksenas, 2011) in verbal abilities, a traditionally female-favoring domain, have been reported in hormonal contraceptives' users with respect to naturally cycling women. Likewise, regarding spatial abilities, a male-favoring domain, several studies showed no significant differences between hormonal contraceptives' users and non-users (Gogos, 2013; Islam et al., 2008; Mordecai et al., 2008; Rosenberg and Park, 2002), although recent studies found that hormonal contraceptives affect mental rotation performance, inducing opposite effects depending on the different progestin (androgenic or anti-androgenic) used (Griksiene et al., 2018; Griksiene and Ruksenas, 2011; Wharton et al., 2008), or on the different dose of EE (Beltz et al., 2015).

The few studies in animals on the effect of hormonal contraceptives on cognitive functions showed dissimilar results in the different experimental paradigms used to test learning and memory. We applied the Morris water maze test, the most widely used paradigm to assess spatial learning and memory performance in rodents (Morris, 1984) to investigate the effects of long-term treatment with EE-LNG (30–125 μg/rat, respectively) in female rats. EE-LNG treatment did not alter spatial learning during the training trials, as well as memory performance during the probe trial (Santoru et al., 2014). Accordingly, the EE-LNG combination was also ineffective in the novel object context recognition and novel object placement recognition tests, two additional tests of spatial memory in rodents. Only the administration of LNG alone (20 μg) was able to induce a greater degree of learning in the novel object context recognition test, but not in the novel object placement recognition test, when compared to the vehicle-treated group (Simone et al., 2015). These results were somehow unexpected given that spatial learning and memory performance have been shown to be influenced by gonadal hormones and by allopregnanolone (Chesler and Juraska, 2000; Johansson et al., 2002), whose levels are blunted following EE-LNG treatment. Moreover, an impairment in recognition memory is induced by lower doses of EE (10 μg), LNG (20 μg), and their combination chronically administered to female Sprague-Dawley rats subjected to the novel object recognition test. By contrast, the higher dose of EE (30 μg) increased preference for the novel object, indicative of a better memory performance compared to vehicle-treated rats (Simone et al., 2015). Interestingly, impaired learning induced by EE and LNG in the novel object recognition test was accompanied by a decrease in hippocampal mRNA expression of BDNF (Simone et al., 2015), the neurotrophic factor involved in learning and memory processes (Bekinschtein et al., 2014).

Another preclinical study suggests that hormonal contraceptives may have long-term detrimental effects on cognition in middle age ovariectomized rats, a rodent model of menopause. In fact, adult intact rats treated with the progestin medroxyprogesterone acetate from months 4 to 8, ovariectomized on month 10 and tested on month 12, following an additional treatment with either vehicle or medroxyprogesterone acetate (meant to mimic hormone replacement therapy at this time point), showed impaired working memory in the water radial arm maze, but no alterations in spatial reference memory in the Morris water maze (Braden et al., 2011). Although this is the only preclinical evidence of a long-lasting effect of hormonal contraceptives, these results might have translational relevance for the present generation of women undergoing hormone replacement therapy in menopause, who is more likely to have used hormonal contraceptives for contraceptive purposes; however, additional supporting evidence for its relevance for women is required.

Finally, chronic administration of EE also impaired working memory in the water radial arm maze, without altering spatial reference memory in the Morris water maze in adult ovariectomized rats (Mennenga et al., 2015). While these results may be relevant to our understanding of cognitive behavior following exposure to estrogens, we believe they are not of translational value with respect to use of hormones for contraceptive purposes, because women using hormonal contraceptives have an intact HPG axis.

Taken together, the preclinical studies in animals are few, and the results are contradictory and inconsistent, implying that more experiments should be performed to shed light on the effects of hormonal contraceptives on cognitive function in rodents.

4.3. Social behavior

Among the effects of long-term treatment with hormonal contraceptives on behavior, we reported changes in social interaction in adult female rats. Specifically, long-term EE-LNG treatment (30–125 μg/rat, respectively) decreases the frequency and duration of dominant and agonistic behaviors in the resident-intruder paradigm (Santoru et al., 2014). In this test, agonistic behaviors (follow the intruder, dominant postures and attacks) delivered by the resident rat towards an intruder are scored to estimate dominance and aggressive social interaction. Thus, our results indicate that EE-LNG-treated rats were less dominant compared to vehicle-treated rats. In agreement, hormonal contraceptives increased frequency of contact aggressions received and relaxed vigilance in cynomolgus monkeys (Henderson and Shively, 2004). Steroid hormones mediate social and aggressive interactions both in animals and humans (Soma et al., 2008). Aggressive behavior and attacks are related to testosterone concentrations in males (Koolhaas et al., 1980); likewise, aggression in female rats varies throughout the ovarian cycle and is blunted by ovariectomy (Ho et al., 2001), suggesting that estradiol and progesterone contribute to this behavior. In addition, allopregnanolone also modulates agonistic behavior by increasing aggressive behaviors in male mice subjected to the resident-intruder test (Fish et al., 2001). Given that long-term EE-LNG treatment, at the same dose and timing used in the behavioral test, decreased brain and plasma concentrations of this neuroactive steroid (Follesa et al., 2002; Porcu et al., 2012; Santoru et al., 2014), we speculated that the reduction in dominant and agonistic behaviors displayed by EE-LNG-treated rats is associated with blunted allopregnanolone levels (Santoru et al., 2014). However, other experimental conditions in which allopregnanolone levels are decreased, are associated with an opposite outcome in the resident-intruder test. In fact, a single exposure to estradiol on the day of birth, which induces defeminization in adult female rodents and markedly and persistently decreases brain and plasma allopregnanolone levels (Calza et al., 2010; Locci et al., 2017), increased agonistic behavior in adult female rats (Berretti et al., 2014). In addition, juvenile social isolation also decreased brain allopregnanolone levels, similar to the EE-LNG treatment, but in male rats and mice it induces an aggressive behavior, rather than a reduction in dominance (Pinna et al., 2003; Serra et al., 2007), suggesting that other factors may influence agonistic behavior. Indeed, the decrease in social dominance in EE-LNG-treated rats might also be related to the decrease in testosterone levels observed in the cerebral cortex of these females (Santoru et al., 2014). In fact, testosterone levels in humans and animals have been found to be associated with aggressive behavior and social dominance (Archer, 2006; Van de Poll et al., 1988). Thus, multiple neuroactive steroids may be influencing agonistic behavior in EE-LNG-treated female rats. The translational relevance of these findings is not clear; in fact, to our knowledge, no evidence is available in women on an impact of hormonal contraceptives on sociability.

Long-term treatment with EE-LNG also induced a decrease in social investigation in female rats subjected to the resident-intruder test (Santoru et al., 2014). Specifically, both body investigation and anogenital investigation were decreased in EE-LNG-treated rats, compared to vehicle-treated rats (Santoru et al., 2014). Anogenital investigation is believed to represent an appetitive behavior that serves to establish, maintain and promote sexual interaction (Everitt, 1990). Thus, the reduced social investigation in EE-LNG-treated rats may represent a decrease in sexual interest toward the intruder. Indeed, long-term treatment with EE-LNG decreased proceptive behaviors (ear wiggling, hops and darts) displayed by receptive females in order to attract and facilitate the male mounts (Santoru et al., 2014).

4.4. Sexual behavior

Hormonal contraceptives have been found to affect women's libido with mixed results ranging from improvement, worsening, or no change in sexual well-being. Some women reported an improvement in sexual well-being, likely the consequence of increased feeling of security (Guida et al., 2005; Strufaldi et al., 2010); others reported a decrease in libido, sexual arousal, and frequency of sexual activities (Battaglia et al., 2012; Caruso et al., 2004; Graham et al., 2007, 1995; Wallwiener et al., 2010), and negative changes in sexuality and mood were the strongest predictors for drug discontinuation in the first six months of hormonal contraceptives' use (Sanders et al., 2001).

The decrease in neuroactive hormones induced by hormonal contraceptives may contribute to their effects on sexual well-being. In fact, in animals both estrogen and progesterone synergistically promote sexual behavior (Gilman and Hitt, 1978), and allopregnanolone also modulates this behavior by increasing proceptive and receptive behaviors in female rats (Frye et al., 1998). Thus, the decrease in estradiol, progesterone and allopregnanolone concentrations induced by hormonal contraceptives may affect sexual behavior in female rats. In agreement, we showed that long-term treatment with EE-LNG (30–125 μg/rat, respectively) markedly decreased the frequency of proceptive behaviors (ear wiggling, hops and darts) in receptive female rats; receptive behaviors (lordosis) were also reduced, although this effect did not reach significance (Santoru et al., 2014). Likewise, hormonal contraceptives decreased proceptive behaviors in non-human primate females that show a reduction in the ability to attract mates and in the cyclic increase in female anogenital swelling (Baum, 1983; Guy et al., 2008; Nadler et al., 1992).

We further tested whether administration of estradiol and/or progesterone could reinstate sexual behavior in EE-LNG-treated rats.

Table 2
Summary of the studies in animals on the effects of hormonal contraceptives on neurotransmission and behavior.

	Effect	Steroid formulation	References
Neurotransmitters			
Serotonin and Tryptophan	Increased brain levels	EE, Medroxyprogesterone acetate, Norethisterone acetate	Baker et al. (1977), Daabees et al. (1981)
Dopamine	Reduced brain levels	Mestranol, Lynestrol, Norethindrone, Norethynodrel	Jori and Dolfini (1976)
Acetylcholine	No effect	EE, Medroxyprogesterone acetate, Norethisterone acetate	Daabees et al. (1981)
Glutamate	No effect	EE, Medroxyprogesterone acetate, Norethisterone acetate	Daabees et al. (1981)
GABA	Increased brain levels	EE, Medroxyprogesterone acetate, Norethisterone acetate	Daabees et al. (1981)
Enzymes			
MAO	Inhibited activity	EE, Norgestrel	Marchi and Cugurra (1974)
Tyrosine hydroxylase	Decreased protein levels	EE, LNG, EE-LNG	Simone et al. (2015)
Choline acetyl transferase	No effect on activity	Mestranol, Lynestrol, Norethindrone, Norethynodrel	Ladinsky et al. (1976)
Receptors			
$GABA_A$	Increased γ2 subunit expression	EE-LNG, LNG	Follesa et al. (2002), Sassoè-Pognetto et al. (2007), Porcu et al. (2012)
Neuropeptides			
BDNF	Decreased expression	EE, EE-LNG	Simone et al. (2015)
Galanin	Increased expression	EE, EE-LNG	Simone et al. (2015)
Behavioral tests			
Elevated plus maze	Anxiety-like behavior	EE-LNG, LNG	Follesa et al. (2002), Porcu et al. (2012)
Elevated plus maze	Anxiolytic-like behavior	EE, EE-LNG	Simone et al. (2015)
Burying behavior test	Anxiety-like-behavior (acute)	LNG, Norethisterone	Picazo et al. (1998)
Burying behavior test	Anxiolytic-like behavior	EE, EE-LNG	Simone et al. (2015)
Locomotor activity	No difference	EE, LNG, EE-LNG	Simone et al. (2015)
Sucrose preference	No depressive-like behavior	EE, LNG	Santoru et al. (2014)
Forced swim test	No depressive-like behavior	EE, LNG	Santoru et al. (2014)
Fear conditioning	Impaired extinction recall	LNG	Graham and Milad (2013)
Morris water maze	No difference in learning and memory	EE, LNG	Santoru et al. (2014)
Novel object recognition	Impaired learning	EE, LNG, EE-LNG	Simone et al. (2015)
Novel object context recognition	No difference in learning and memory	EE, LNG, EE-LNG	Simone et al. (2015)
Novel object placement recognition	No difference in learning and memory	EE, LNG, EE-LNG	Simone et al. (2015)
Resident-intruder	Decreased dominant behavior; Decreased social investigation	EE-LNG	Santoru et al. (2014)
Sexual activity	Decreased proceptive behavior; No difference in lordosis	EE-LNG	Santoru et al. (2014)

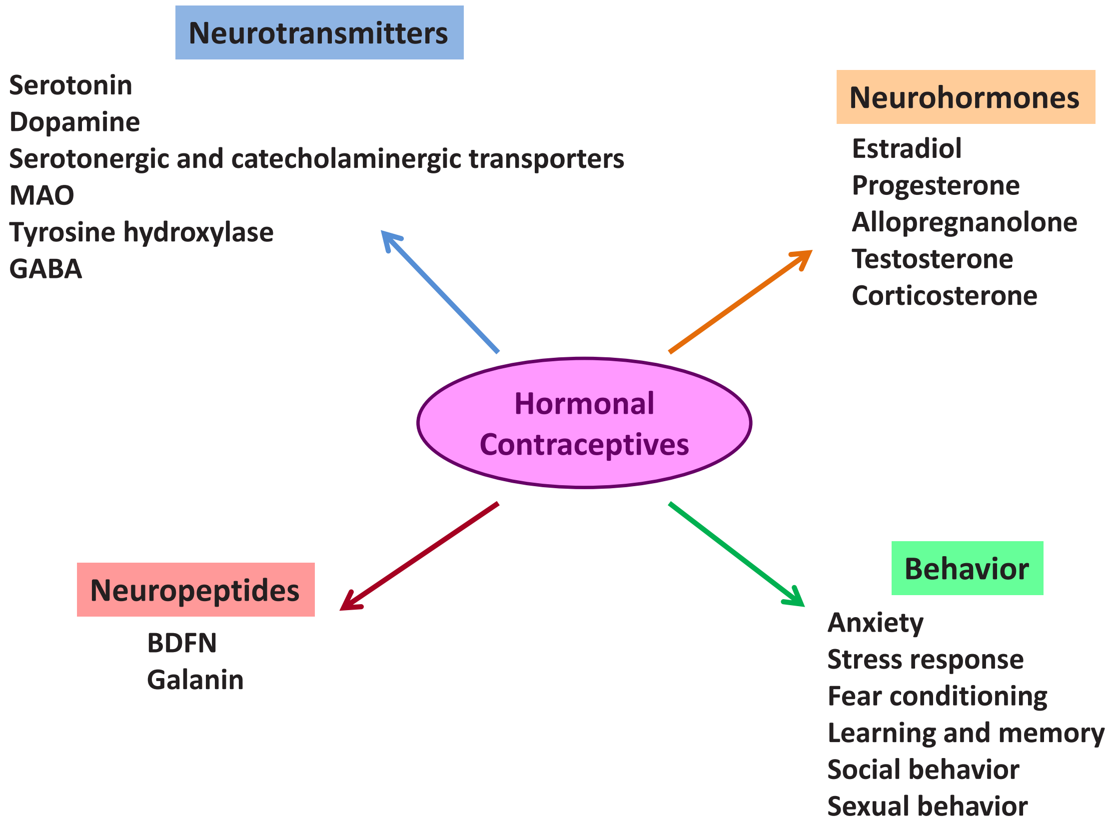


Fig. 2. Schematic representation of hormonal contraceptives' influences on several aspects of brain function, including neurotransmitters, neuropeptides, steroid hormones and neurosteroids, and related behaviors.

Administration of estradiol alone did not influence lordosis and proceptive behaviors, while progesterone, alone or in combination with estradiol, restored such behaviors in EE-LNG-treated female rats, suggesting that the marked decrease in brain and peripheral levels of progesterone, induced by long-term treatment with EE-LNG, contributes to the reduced sexual behavior in female rats (Santoru et al., 2014).

Several studies suggest that effects of progesterone on sexual behavior may be mediated by its metabolite allopregnanolone. In fact, systemic administration or intracerebral infusion of this neuroactive steroid promotes mating in ovariectomized rats (Frye and Rhodes, 2006; Frye and Vongher, 2001; Glaser et al., 1985; Henderson, 2007). Further, brain allopregnanolone levels are increased when rats are maximally receptive or following mating (Frye, 2001; Glaser et al., 1985). By contrast, midbrain infusion of inhibitors of the biosynthetic enzymes that convert progesterone to allopregnanolone, attenuates sexual behavior in female rats (Frye and Vongher, 2001). In agreement, we found that administration of finasteride (a 5α-reductase inhibitor that blocks the conversion of progesterone to allopregnanolone), which *per se* did not alter the lordosis quotient and the frequency of proceptive behaviors, prevented the reinstatement of proceptive behaviors induced by progesterone administration in EE-LNG-treated rats, suggesting that allopregnanolone plays a major role in such behaviors and that the reduction in proceptive behaviors induced by EE-LNG treatment is related to the decrease in the brain concentrations of this neuroactive steroid (Santoru et al., 2014).

Testosterone also affects sexual behavior in both animals and humans (Frye, 2001; Giraldi et al., 2004; Stuckey, 2008), and hormonal contraceptives reduce serum testosterone levels in women (Graham et al., 2007; Paoletti et al., 2004), as well as its cerebrocortical concentrations in female rats (Santoru et al., 2014). However, administration of progesterone, finasteride or their combination did not alter cerebrocortical testosterone levels in EE-LNG-treated rats, suggesting that this hormone does not contribute to the reduction in proceptive behaviors induced by long-term EE-LNG treatment (Santoru et al., 2014).

Overall, these results suggest that the decrease in allopregnanolone concentrations, induced by hormonal contraceptives, might contribute to those negative changes in sexual well-being sometimes reported by women.

4.5. Reward

Ovarian hormones are known to influence reward in animals and humans. Dopamine release varies across the estrus cycle, being increased during proestrus and early estrus when endogenous estradiol peaks, and both estradiol and progesterone modulate reward-related behavior in rodents (Yoest et al., 2018). Allopregnanolone also exerts rewarding effects in animals (Finn et al., 1997; Fish et al., 2014; Sinnott et al., 2002), and modulates alcohol and cocaine consumption (Anker and Carroll, 2010; Morrow et al., 2006). Likewise, hormonal fluctuations across the menstrual cycle influence reward reactivity in women (Bayer et al., 2013; Dreher et al., 2007). Further, hormone therapy in perimenopausal women increased the activity of the reward system (Thomas et al., 2014), while a pharmacologically-induced decline in levels of ovarian hormones reduced amygdala responsivity to monetary reward in healthy premenopausal women (Macoveanu et al., 2016). Given that hormonal contraceptives alter endogenous levels of estradiol, progesterone and allopregnanolone, it is likely that they may also affect reward. However, very few studies are available in women that lead to inconclusive results as, hormonal contraceptives were reported to increase sensitivity to monetary reward (Bonenberger et al., 2013), but also to suppress the oxytocin-induced reward associated to the partner's attractiveness (Scheele et al., 2016). Further, while a single systemic administration of estrogens and/or progesterone has been shown to affect reward in rodents (Anker and Carroll, 2010; Yoest et al., 2018), to the best of our knowledge, no preclinical studies are available yet on the effects of long-term treatment with hormonal contraceptives on reward-related behavior. Thus, future studies, both in animals and women, are warranted to investigate the effects of hormonal contraceptives on reward.

5. Conclusions

Taken together, the reported evidence suggests that hormonal contraceptives influence several aspects of brain function, including neurohormones, neurotransmitters, neuropeptides, learning and memory, and emotional, social, agonistic and sexual behaviors (Table 2 and Fig. 2). However, the existing literature on animal studies is limited, with different protocols employed that lead to varied results. Thus, much work remains to be done to investigate the neurobiological mechanisms underlying the action of hormonal contraceptives on the brain. Hormonal contraceptives may increase vulnerability to develop certain psychiatric disorders; hence, evaluation of their actions in animal models in which insertion of alleles associated to psychiatric illness (mineralocorticoid receptor haplotypes, BDNF polymorphism, etc.) may enable a mechanistic investigation of the potential high vulnerability of some hormonal contraceptives' users in a way that cannot be pursued in humans. In conclusion, future research in understanding the neurobiological effects of hormonal contraceptives is needed to improve women's health and to help women making informed choices on the advantages and disadvantages of hormonal contraception.

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