European Neuropsychopharmacology (2015) 25, 113–123

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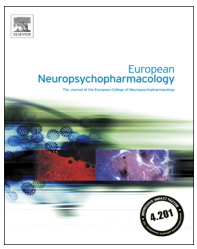

Elevation of brain allopregnanolone rather than 5-HT release by short term, low dose fluoxetine treatment prevents the estrous cycle-linked increase in stress sensitivity in female rats

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Received 22 April 2014; received in revised form 21 November 2014; accepted 24 November 2014

KEYWORDS
Estrous cycle;
Anxiety;
Periaqueductal grey matter;
Fluoxetine;
Allopregnanolone;
c-fos

Abstract
Withdrawal from long-term dosing with exogenous progesterone precipitates increased anxiety-linked changes in behavior in animal models due to the abrupt decrease in brain concentration of allopregnanolone (ALLO), a neuroactive metabolite of progesterone. We show that a withdrawal-like effect also occurs during the late diestrus phase (LD) of the natural ovarian cycle in rats, when plasma progesterone and ALLO are declining but estrogen secretion maintains a stable low level. This effect at LD was prevented by short-term treatment with low dose fluoxetine.

During LD, but not at other stages of the estrous cycle, exposure to anxiogenic stress induced by whole body vibration at 4 Hz for 5 min evoked a significant decrease in tail flick latency (stress-induced hyperalgesia) and a decrease in the number of Fos-positive neurons present in the periaqueductal gray (PAG). The threshold to evoke fear-like behaviors in response to electrical stimulation of the dorsal PAG was lower in the LD phase, indicating an increase in the intrinsic excitability of the PAG circuitry. All these effects were blocked by short-term administration of fluoxetine (2×1.75 mg kg^{-1} i.p.) during LD. This dosage increased the whole brain concentration

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http://dx.doi.org/10.1016/j.euroneuro.2014.11.017

of ALLO, as determined using gas chromatography–mass spectrometry, but was without effect on the extracellular concentration of 5-HT in the dorsal PAG, as measured by microdialysis.
We suggest that fluoxetine-induced rise in brain ALLO concentration during LD offsets the sharp physiological decline, thus removing the trigger for the development of anxiogenic withdrawal effects.

1. Introduction

Premenstrual syndrome (PMS) and its more severe counterpart: premenstrual dysphoric disorder, blights the lives of millions of women worldwide (Steiner, 1997). In susceptible women the symptoms, which include angry outbursts, irritability and anxiety (ACOG, 2001), can be considered an exaggerated and inappropriate response to everyday acute stressful challenges. Surprisingly, given the enormity of the problem of PMS, there has been relatively little basic scientific study into its neurophysiological basis. What is clear from clinical studies is that PMS is dependent on cyclical variations in female sex hormones. Symptoms do not appear in anovulatory cycles (Backstrom et al., 2003). However ovulation itself is not the key factor since many women taking the combined contraceptive pill on a 21 day on, 7 day off dosing regimen, which prevents ovulation, also experience PMS-like symptoms, which peak during the 7 day drug free period (Kadian and O'Brien, 2012). In both cycling women and those taking the pill, symptoms occur at a time when blood levels of progesterone and estrogen, or their synthetic analogues, are in rapid decline.

In rats, withdrawal from long term dosing with exogenous progesterone at doses sufficient to raise progesterone to the high physiological range, precipitates increased anxiety (Smith et al., 1998, 2006). Spontaneously cycling rats in the late diestrus phase, when progesterone secretion declines sharply but estrogen secretion remains at a stable low level (Butcher et al., 1974), also become more fearful in an open field arena (Devall et al., 2009). Moreover, exposure of rats to 5 min of mild anxiogenic vibration stress (5 min vibration at 4 Hz whilst confined in a tube, Jørum, 1988) during late diestrus evokes a hyperalgesia, which is not seen when the animals are exposed to the same stressor at other stages of the cycle (Devall et al., 2009). These findings suggest that falling progesterone predisposes to an enhanced response to psychogenic stress. By analogy, the rapid decline in ovarian progesterone secretion in women during the late luteal phase might also provide a trigger for the enhanced responsiveness to psychogenic stress, which is a feature of the premenstrual period in many women (Nillni et al., 2011; Hoyer et al., 2013; Gollenberg et al., 2010). Thus whilst the cause of PMS in women is likely to be multifactorial, withdrawal from progesterone could be a significant contributory precipitating factor.

Progesterone passes readily through the blood brain barrier. The active agent that triggers the neuronal response to progesterone withdrawal in female rats is not however the native steroid hormone, but its neuroactive metabolite allopregnanolone (ALLO: 5α-pregnan-3α-ol-20-one or 3α,5α-tetrahydroprogesterone) (Gulinello et al., 2003; Smith et al., 1998). This progesterone metabolite ALLO is a potent positive allosteric modulator of the actions of GABA at $GABA_A$ receptors (Paul and Purdy, 1992) and its concentration in random cyclic female rat brain correlates with that of its precursor progesterone in plasma (Corpéchot et al., 1993). Ovarian secretion of both progesterone and ALLO decreases sharply at late diestrus (Ichikawa et al., 1974; Holzbauer, 1975), and so the concentration of ALLO in the brain will decrease in parallel. There is some endogenous production of progesterone and ALLO in the male rat brain (Cook et al., 2014). A similar situation appears to exist in the female rat, as evidenced by the persistence of these steroids in the brains of ovariectomised and adrenalectomised animals, but this is at concentrations around 9-fold and 40-fold lower, respectively, than those seen in the brains of intact random cycling rats (Corpéchot et al., 1993). Even in female rats at late diestrus, when ovarian progesterone secretion is low, brain concentrations of ALLO and progesterone were 10-fold higher than in males (Fry et al., 2014).

Whether naturally during the ovarian cycle or following administration of exogenous progesterone, withdrawal from ALLO, triggers upregulation of extrasynaptic $GABA_A$ receptors in the brain and consequent changes in excitability of neuronal circuits associated with anxiety (Gangisetty and Reddy, 2010; Griffiths and Lovick, 2005a, 2005b; Gulinello et al., 2003; Lovick et al., 2005; Smith et al., 1998). The dynamic of the fall in brain concentration of progesterone appears critical because abrupt withdrawal from an exogenous progesterone-dosing regimen in rats precipitates an increase in responsiveness to anxiogenic stressors, whilst a gradually tapered withdrawal does not (Doornbos et al., 2009; Saavedra et al., 2006). In this respect it is interesting that in women, an association between clinical features of PMS and rate of decrease in progesterone during the luteal phase has been noted (Halbreich et al., 1986). Thus we reasoned that if the sharp fall in brain ALLO concentration that occurs at the end of the estrous cycle in rats is a trigger for the development of increased stress sensitivity, measures to produce a more gradual reduction in brain concentration of ALLO at the end of the estrous cycle should prevent the development of withdrawal-like symptoms.

In male rats and mice, the antidepressant fluoxetine (FLX) has been shown to induce an increase in the concentration of ALLO in the brain, which can be detected within 20 min of acute administration (Pinna et al., 2009; Serra et al., 2001; Uzunov et al., 1996). We therefore hypothesized that if FLX produces a similar effect in female rats in late diestrus, then short term dosing with FLX during this stage of the ovarian cycle should offset the rapid physiological fall in the concentration of ALLO. Further, that the stimulus for the development of withdrawal effects, which normally charac-