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Progesterone and anxiety during the oestrus cycle in rats genetically selected for high and low active avoidance

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Abstract. In this research, the changes of anxiety and blood progesterone levels during the oestrus cycle were studied in rats genetically selected for high (KHA) and low (KLA) acquisition of active avoidance. Anxiety levels were measured by the time spent in open arms of the elevated plus-maze. Progesterone levels were determined by radioimmunoassay. KLA rats exhibited no significant changes in anxiety levels during the oestrus cycle. KHA rats showed a significant variation of anxiety during the oestrus cycle with a high level in the diestrus phase and a low level in proestrus. Moreover, anxiety in diestrus in KHA rats was higher than in KLA rats. Additionally, increased progesterone levels were observed in KLA rats in comparison with the KHA strain, during both diestrus and proestrus. Anxiety levels corresponded to plasma progesterone during the oestrus cycle in both rat strains.

Keywords: progesterone, anxiety, oestrus cycle, active avoidance, coping style.

Progesterone is a sex steroid hormone associated with female reproductive functions, including sexual behaviour, uterus preparation for embryo implantation and maintenance of pregnancy. This hormone is mainly synthesized in the ovaries and placenta, but it is also produced by the adrenal cortex and the central nervous system (CNS) of both male and female mammals (Gutai et al. 1977; Mensah-Nyagan et al. 1999; Tuckey 2005). The fact that both males and females synthesize this hormone indicates that its functions are not limited to the female reproductive physiology. For example, progesterone regulates various non-reproductive functions in the CNS related to neurogenesis, neuroprotection, neural circuit organisation, oligodendrogenesis, myelination, neuronal plasticity, and mood (Snyder, Hull 1980; Schumacher et al. 2017). Therefore, given that progesterone is synthesised, metabolised and exerts its functions in the CNS, it is referred to as a neurosteroid.

Neurons and glial cells in the brain can synthesise progesterone *de novo* from cholesterol as they express the enzymes responsible for its synthesis and metabolism (Testas et al. 1989; Mellon, Deschepper 1993; Schumacher et al. 2017). Thereafter, the progesterone resulting from either circulating plasma or CNS local synthesis binds to its specific intracellular and membrane receptors to regulate

the molecular and cellular processes underlying the brain functions.

We studied plasma progesterone levels in female rats of two psychogenetically selected strains. Koltushi High Avoidance (KHA) and Koltushi Low Avoidance (KLA) rat strains have been developed based on selective breeding at Pavlov Institute of Physiology from Wistar rats using divergent performances in avoidance conditioning in two-way shuttle-boxes during five consecutive days as the criterion (Ryzhova et al. 1983). KHA and KLA strains of rats demonstrate different levels of anxiety (Zhukov, Vinogradova 1994). In the present study the females of 46th and 47th generation of the selection were used.

Three-month old females were tested in the elevated plus-maze (length of beams — 100 cm, width — 10 cm, and height of closed arms walls — 20 cm) during diestrus and proestrus. Vaginal smears were taken daily during at least three weeks before a behavioural test. Diminished time spent in open arms during five minute plus-maze test was used as a measure of anxiety. Blood samples from tail veins were collected immediately after plus-maze testing.

Samples were assayed radioimmunologically as described previously (Savchenko, Proimina 1986) in duplicate using an antiserum obtained in our laboratory and a tritiated hormone from the State Institute of Applied Chemistry (St Petersburg, Russia). The average intra-assay coefficient of variation was 2.05%, and the average inter-assay coefficients across high and low controls were 4.85%.

Data are presented in Table 1.

In KHA rats both progesterone levels and anxiety levels differed considerably during diestrus and proestrus. In KLA rats there was no significant difference for anxiety levels between the two stages, and the difference for progesterone levels was less pronounced. We found significant interstrain differences for both progesterone and anxiety levels. Progesterone levels were higher and anxiety levels were lower in KLA rats during both stages of the oestrus cycle.

Interstrain differences in progesterone levels may be one of the causes of different maternal behaviour in KHA and KLA rats found earlier (Vinogradova, Zhukov 2004). The latency of the first approach to pups after their removal from the nest has been lower in KHA rats, and they needed more time to return all pups to the nest. The link between blood plasma progesterone levels and maternal behaviour has also been found in humans (Glynn et al. 2017).

The interstrain differences of progesterone variations in the oestrus cycle are in agreement with the types of female infertility in KHA and KLA rats found in preliminary studies. During breeding we encountered problems with infertility during everyday transportation of the animals from the vivarium to the lab. However, the infertility was of a different nature: a permanent anestrus was found in KLA females, while foetal resorption was observed in KHA rats.

The most interesting discovery of our present study is the correspondence between progesterone levels and anxiety: the higher the progesterone the lower the anxiety. As we noted above, progesterone plays a key role in development, differentiation, and diverse reproductive and non-reproductive functions. Particularly in the CNS, where progesterone regulates various functions such as reproductive behaviour (Gómez-Camarillo et al. 2011), learning and memory (Yousuf et al. 2017), neuroprotection (Singh, Su 2013), and mood (Conway et al. 2007; Dichtel et al. 2017; Studd 2014).

Table 1. Blood progesterone level (nM) and time spent in open arms of the plus-maze (s) in KHA and KLA rats during diestrus and proestrus

	KHA		**KLA**	
	Diestrus n = 17	Proestrus n = 14	Diestrus n = 16	Proestrus n = 15
Progesterone	1.2 ± 0.1 **	3.3 ± 0.5	4.3 ± 0.5 * ##	6.2 ± 0.4 ##
Open arms time	44.4 ± 7.0 **	115.4 ± 8.2	118.1 ± 10.0 ##	143.7 ± 10.7 #

* $p < 0.05$; ** $p < 0.01$ — *vs* proestrus (for each rat strain)
# $p < 0.05$; ## $p < 0.01$ — *vs* similar parameter in KHA rats
Mann–Whitney U test

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Progesterone receptors are widely distributed in the brain. A significant number of progesterone receptors has been found in the hippocampus and other emotiogenic structures (Camacho-Arroyo et al. 2017; Meffre et al. 2013).

Progesterone exerts its effects on its target cells through three central pathways, namely the classical (first) and non-classical (second and third) ones (González-Orozco, Camacho-Arroyo 2019). In the classical pathway, progesterone binds to an intracellular receptor, activating such a transcription factor which dimerises and translocates to the nucleus. There, it binds to specific DNA sequences, called progesterone response elements, which are mainly located in the gene promoter regions, thus regulating their expression. In contrast, one of the non- classical pathways involves progesterone receptor ligand-independent activation by membrane-associated kinases and the activation of the multiple G protein-coupled membrane receptors of progesterone, which in turn activates pathways related to cAMP-dependent protein kinase A, Ca^{2+}-dependent protein kinase C, PI3K/Akt and ERK/MAPK. Furthermore, the other non-classical pathway of progesterone action includes the modulation of the gamma-aminobutyric acid receptors of type A ($GABA_A$) following its conversion into allopregnanolone.

Allopregnanolone was recognized as a 5*α*-reduced metabolite of progesterone (Beall, Reichstein 1938). It was named a *neurosteroid* in 1981 by Baulieu's team, who discovered that the brain "acting like a peripheral gland" expresses the enzymatic machinery required to synthesise allopregnanolone *de novo* starting from pregnenolone, the precursor of all neurosteroids (Corpéchot et al. 1981). Allopregnanolone's anti-convulsant, anxiolytic and anti-depressant pharmacological effects after its administration in animals and humans were soon recognised to be mediated by a mechanism of action that includes the fast allosteric modulation of the action of GABA at $GABA_A$ receptors (Majewska et al. 1986; Belelli et al. 2018). The neurophysiological role of allopregnanolone in permitting the fine-tuning and regulation of the strength of $GABA_A$ receptors to agonists, positive allosteric modulators, and GABAmimetic agents, was also unveiled (Pinna et al. 2000). By affecting $GABA_A$ receptors, allopregnanolone also regulates emotional animal behaviour in rodent stress models and humans with posttraumatic stress disorder and major unipolar depression (Rasmusson et al. 2019; Pineles et al. 2018).

The changes in the brain progesterone levels can be independent of blood circulating hormones because of neurosteroidogenesis (Compagnone, Mellon 2000). Yet, obviously, for the regulation of brain functions the peripheral progesterone also is important. For example, in rats anxiety can be induced by progesterone withdrawal (Islas-Preciado et al. 2016), and in women premenstrual syndrome is associated with rapid fall of progesterone synthesis at the end of the menstrual cycle (Bäckström et al. 2014).

The present findings suggest that circulating progesterone may account at least in part for the behavioural differences characterising two strains selected for contrasting learning abilities.

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