Trakia Journal of Sciences, Vol. 12, Suppl. 1, pp 38-41, 2014

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

INFLUENCE OF 17-β-ESTRADIOL ON OPEN FIELD BEHAVIOUR OF IMMATURE FEMALE RATS

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ABSTRACT

PURPOSE: Endogenous gonadal steroids have many effects on the brain throughout the lifespan, beginning during gestation and continuing into senescence. There are data that they seem to play an important role in the open field behavior in adult rodents. The possible influence of the gonadal steroids on sex related determination of brain and behavior during the prematuration has received little attention in the literature. The purpose of the present study was to investigate the influences of 17-β-estradiol on the horizontal and vertical locomotor activity of immature female rats.

METHODS: The experiments were carried out on immature female Wistar rats (age 6 weeks, 90g). 17-β-estradiol (40 μg/100 g b.w.) was administered intraperitoneally once daily for 3 days of the experiment. The changes in the locomotor activity of the rats were evaluated using open field test 4 and 24 hours after injections.

RESULTS: The data received show that exogenous estrogenization of immature female rats (4 and 24 hours after treatment) causes decrease in the horizontal (ambulation) and vertical locomotor activity (rearing) in open field test.

CONCLUSIONS: The obtained results confirmed that estradiol significantly modify open field behavioral responses of female rats during the period of prematuration.

Key words: 17-β-estradiol, open field test, immature female rats

INTRODUCTION

Gonadal steroids have many effects on the brain throughout the lifespan, beginning during gestation and continuing into senescence. These hormones affect areas of the brain that are not primarily involved in reproduction, such as the basal forebrain, hippocampus, caudate putamen, midbrain raphe, and brainstem locus coeruleus (1). Endogenous gonadal steroids seem to play an important role in the open field behaviour and its rhythmicity. They regulate synaptogenesis. There are data for formation of new excitatory synapses induced by estradiol (2, 3). There are developmentally programmed sex differences in the hippocampal structure related to the different behaviour strategies in rats. Ovarian steroids have effects on the brain including brainstem and midbrain catecholaminergic neurons, midbrain serotonergic pathways and the basal forebrain cholinergic system (4). Regulation of the serotonergic system appears to be linked to the presence of estrogen- and progestin- sensitive neurons in the brain raphe, whereas the ovarian steroid influence on cholinergic function involves induction of cholineacetyltransferase and acetylcholinesterase according to a sexually dimorphic pattern. Because of these widespread influences on these various neuronal systems it is not surprising that ovarian steroids produce measurable cognitive

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effects after ovariectomy and during aging (1). Changes of activity of both monoaminergic and amino acid transmitters in the frontal cortex and basal forebrain may contribute to enhancing effects of estrogen on learning and memory. Levels of GAB A are increased following estrogen treatment (5). Bowman et al. (2002) indicate that estradiol decreases anxious behavior on the open field of ovariectomized rats and it may moderate stress effects on cognition and anxiety through both organizational and activation effects (6). The effect of estrogen on the hippocampal muscarinic M4 receptor subtype is also novel finding in this direction (7). The finding of Gureviciene et al. (2003) show, that estrogen treatment increases the number of active hippocampal NMDA-receptors in mouse (8). Excess of estradiol in mature female rats accelerate the acquisition of active avoidance performance, while the lack of estrogens results in amnesia of passive avoidance performance (9, 10).

Because the data about gonadal modulation of non-reproductive behavior of rats during the prematuration are very scant the aim of the present study was to investigate the influences of 17-β-estradiol on open field behavior in immature female rats.

MATERIALS AND METHODS

Animals: Immature female Wistar rats (body weight 90g; age 6 weeks) served as research objects. They were housed in groups of 6 per cage and kept under a normal 12 h light/dark cycle and 21 ± 1°C temperature. Food and water were available *ad libitum.*

The experimental procedures were carried out in accordance with the institutional guidance and general recommendations on the use of animals for scientific purposes.

Open field test (OF): The square open field arena (1m x 1m) is divided in a peripheral and center arena by a grid cross on the floor. The animals were tested in soundproof room only once in the arena for 5 min and summed activity scores (number of crossing squares and number of standing upright) were used as a measured of horizontal (ambulation) and vertical locomotor activity (rearing). Environmental odors were removed after each session to avoid influences on the behavior.

Drugs and treatment: 17-β-estradiol (40 μg/100g, i.p.) was purchased from Sigma Chemical Co., (St. Louis, MO) and was dissolved in propyleneglycol. Rats from experimental group were injected intraperitoneally (i.p.) once a day for 3 days with 17-β-estradiol. The control group was injected i.p. with the same volume of propyleneglicol. Four and 24 hours after injection the animals were tested in open field arena.

Data analysis: The results were statistically assessed by Student's t test. Values are mean ± S.E.M. Values of $P < 0.05$ were considered to indicate statistical significance.

RESULTS

The data received show that estradiol (40 μg/100 g) provokes decrease of the horizontal and vertical locomotor activity in immature female rats during the whole investigated period. On the 1st and 3rd experimental days, 4 or 24 hrs after injection of hormone, ambulation was significantly reduced compared to the control ($P<0.05$). Although rearing was reduced during the three days of the experiment, the effect was more pronounced on the 1st day 24hrs after estradiol application ($P<0.001$) **(Figure 1).**

Four hours after injection estradiol significantly decreased exploratory (35%, 30% and 49%) and rearing (38%, 28% and 44%) activity in open field test vs. control during the three experimental days. The same effect was observed 24 hrs after estradiol application – reduction of ambulation (38%, 21% and 19%) and rearing (52%, 35% and 26%) during the 3 days vs control for three days respectively **(Figure 2).**

DISCUSSION

Taking into consideration the obtained data we could suggest that the relative changes of ambulation and rearing compared to the control after exogenous estrogenization of female immature rats during the three days of the experiment in open field test are in relation to the increased sensitivity of $GABA_A$-receptors or to interaction with 5HT-receptors or are due to endocrine status modulation.

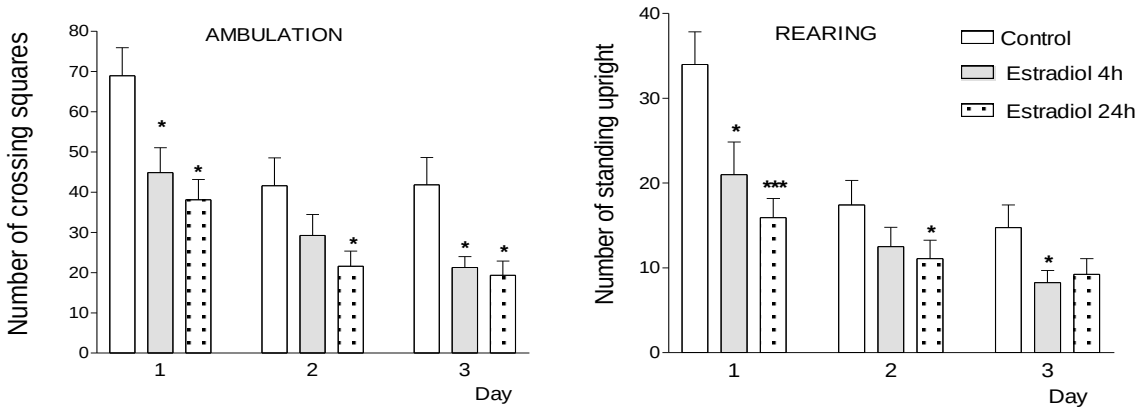

Figure 1. Ambulation and rearing activity in female immature rats tested in open field 4 and 24 hours after i.p. injection of 17-β-estradiol (40 μg/l00 g b.w.). Data are presented as mean + S.E.M.; *P<0.05, ***P<0.001 vs. control.

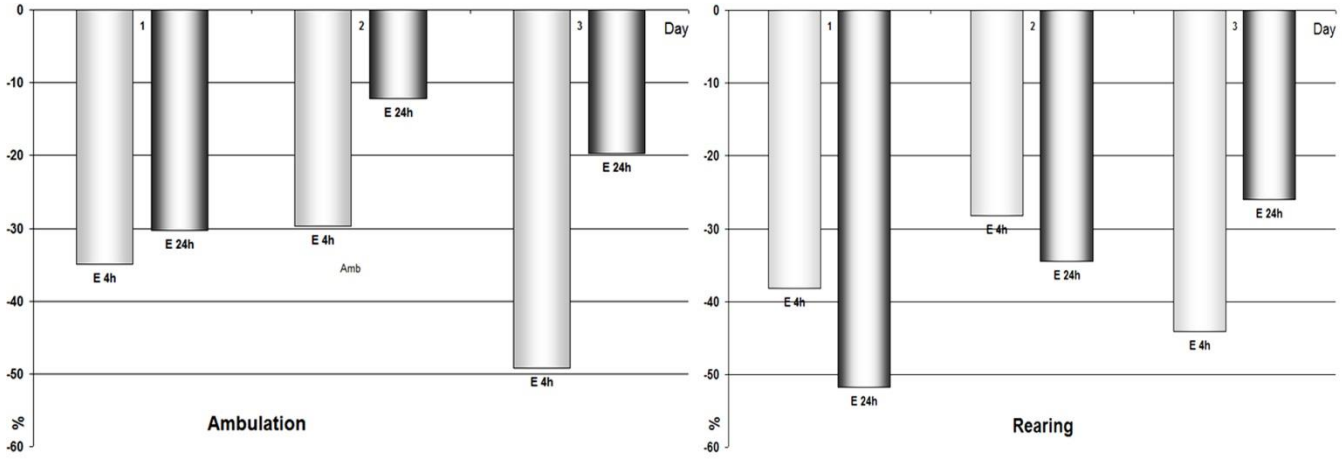

Figure 2. The relative changes of ambulation and rearing by exogenous estrogenization (effect of estradiol after 4hrs -E4h and after 24hrs – E24h) compared to the control during the three days of the experiment. Data are presented as percentage % = [test/control] x 100 -100%.

The basic neural networks that generate rhythmic locomotor movements in vertebrates are present in the spinal cord at very early stages in ontogeny, and these preexisting circuits are then modified during development to perform more complex adult locomotor functions. Current research on the mechanisms responsible for the maturation of early locomotor circuits suggest that the descent of modulatory brainstem control systems may be particularly important for restructuring immature spinal networks so that they impart greater flexibility and control of their motor output (2). Immature networks are precursors of adult locomotor circuity.

Addressing the developmental mechanisms responsible for the maturation of locomotor networks is difficult because changes occur at several levels: the electrical properties of component neurons in locomotor circuits change during development, various transmitter receptors on spinal neurons are expressed according to a specific developmental timetable and their properties can be modified during development and even the neuronal composition of developing networks may change (2).

Developmental changes in different spinal transmitter receptors are described (11, 12). For instance, the NMDA-glutamate receptor (which allows calcium entry into neurons) reportedly change in the course of development such that calcium influx is enhanced in younger neurons (14). The sensitivity of glutamate receptors in embryogenesis to various agonists increases with the age (12) and only later become hyperpolarizing (11, 15). It is possible GABA neurons may change their transmitter phenotype (from GABA to

glutamate) at a particular stage in development (10).

The role of steroids (for modulation of the functions of CNS through their non-genomic effect on $GABA_A$-receptors) in these complicated conditions is very important. It is in regional (16) and development dependence. Some of these mechanisms probably have a place in steroid modulation of ambulation and rearing of immature female rats by exogenous estrogenization.

In summary, the current study provides novel information showing that estradiol significantly decreased open field behavioral responses of female rats during the period of prematuration. These effects can not be fully explained by the known mechanisms at present.

Further studies are needed to clarify the primary site and mechanism of these actions, using various methods and animal models.

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