Neurosci Bull June 1, 2015, 31(3): 288–296. http://www.neurosci.cn
DOI: 10.1007/s12264-014-1510-8

·Original Article·

Effects of testosterone and estradiol on anxiety and depressive-like behavior *via* a non-genomic pathway

Barbora Filova[1], Maria Malinova[1], Janka Babickova[1,5], Lubomira Tothova[1,5], Daniela Ostatnikova[2], Peter Celec[1,3,4,5], Julius Hodosy[1,2,5]

[1]*Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia*

[2]*Institute of Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia*

[3]*Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia*

[4]*Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia*

[5]*Center for Molecular Medicine, Slovak Academy of Sciences, Bratislava, Slovakia*

Corresponding author: Julius Hodosy. E-mail: hodosy@gmail.com

© Shanghai Institutes for Biological Sciences, CAS and Springer-Verlag Berlin Heidelberg 2015

ABSTRACT

Besides their known slow genomic effects, testosterone and estradiol have rapid effects in the brain. However, their impact on mood-related behavior is not clear. The aim of this study was to investigate the non-genomic pathway of testosterone and estradiol in the amygdala in relation to anxiety and depressive-like behavior. Sham-operated and gonadectomized male rats (GDX) supplemented with testosterone propionate, estradiol, or olive oil were used. Five minutes after administration, anxiety and depression-like behavior were tested. Estradiol increased anxiolytic behavior in the open-field test compared to the GDX group, but administration of testosterone had no significant effect. Besides, c-Fos expression in the medial nucleus of the amygdala significantly increased after testosterone treatment compared to the GDX group, while no significant difference was observed in the central and the basolateral nuclei of the amygdala in the testosterone-treated group compared to the GDX group. In conclusion, estradiol had an anxiolytic effect *via* a rapid pathway, but no rapid effect of testosterone on anxiety was found. Further studies elucidating whether the rapid effect is mediated by a non-genomic pathway are needed.

Keywords: non-genomic effects; steroids; anxiety; depression; c-Fos; amygdala

INTRODUCTION

The effects of sex hormones are largely mediated by intracellular receptors. Steroid hormone receptors belong to a large group of ligand-activated transcription factors that bind to specific DNA sequences and modulate the transcription of genes encoding a wide variety of proteins. These genomic effects of sex hormones are relatively slow and usually appear after one hour to several days[1]. The genomic mechanism seems to play a role in the long-term effects of hormones on tissues sensitive to steroids, such as the brain. The effects of steroids on the nervous system are limited to cells that contain intracellular steroid receptors. Moreover, the effects of these receptors may also induce other processes, for example, induction of key metabolic enzymes of neurotransmitter metabolism[2].

Apart from the slow genomic effects of steroids, rapid effects have also been described. These effects are too fast to activate DNA transcription and translation (within a few seconds to minutes). Therefore, they are also referred to as non-genomic effects[3]. Studies of *in vivo* and *in vitro* models have shown that nuclear receptors, which are activated by binding to hormones, interact with other transcription factors without direct binding to DNA[3]. The

non-genomic activity of steroids causes the rapid induction of conventional second-messenger signal transduction cascades, including a rapid increase in intracellular calcium concentration and the activation of protein kinase A, protein kinase C, and mitogen-activated protein kinase[4]. The induction of secondary messengers *via* the non-genomic effects of steroids is insensitive to transcription and translation inhibitors.

Testosterone, its metabolite 5α-dihydrotestosterone, and estradiol affect anxiety, depressive-like behavior, and memory. Results from animal studies suggest that testosterone has antidepressant[5, 6] and anxiolytic effects[6, 7]. Carrier and Kabbaj found that testosterone significantly decreases depressive-like behavior of male rats in forced swim tests. Since behavioral despair is reversed by estradiol benzoate but not dihydrotestosterone, the authors concluded that this effect is probably mediated by the aromatization of testosterone to estradiol[5]. Similar results have been found in experiments with mice. Older male mice spend more time immobile than older females. Depressive-like behavior is reduced in both sexes compared to controls upon receiving testosterone, dihydrotestosterone, 5α-androstane-3α,17β-diol, and estradiol[8].

The anxiolytic effects of testosterone are well known in rats[9] and mice[10]. However, the underlying mechanism is not well understood. Removal of the primary source of endogenous androgens (castration) in male rats increases anxiety behavior in the elevated plus maze and open-field tests[11]. In contrast, repeated injections of testosterone into castrated rats reduce anxiety behavior in probe-burying tests[12], but this behavior does not occur after administration of 3α-androstanediol and 5α-androstane-3α, 17β-diol (3α- and 5α-reduced metabolites of testosterone, respectively).

Estradiol has positive effects on reference memory in aging mice[13] and can decrease anxiety and depressive-like behavior[14]. However, the results from various studies are in conflict. For example, in adult female meadow voles, estradiol impairs spatial performance and increases depressive-like behavior[15]. Treatment of mice with estradiol benzoate increases anxiety in the open-field, elevated plus maze, and light-dark transition tests, as well as activity in running wheels, compared to vehicle administration[16]. In contrast, Walf and Frye reported that acute administration of estradiol in older female mice has greater anti-anxiety effects in the open-field, mirror chamber, and light-dark transition tests, and anti-depressant behavior in the forced-swim task compared to vehicle administration. Also, application of estradiol to the hippocampus in rats for 10 min following training improves performance in hippocampus-mediated tasks[17]. These inconsistencies may be caused by differences in the doses of estradiol, duration of exposure, and activity of the hypothalamic-pituitary-adrenal axis[14], or by unclear differences between the genomic and non-genomic effects of steroid hormones[6].

Steroids affect neurons, glial cells, sexually dimorphic nuclei, and soma size in various brain regions, including the amygdala and hippocampus[18]. Rapid effects of steroids on brain structures can be visualized by c-Fos immunostaining. The c-Fos protein is a suitable marker for labeling activated neurons in response to different experimental stimuli such as growth factors, ion channel activation, neurotransmitter release, and behavior. The c-Fos gene can be used to easily recognize activated neurons *in vivo* after delivering stimuli such as drugs, or after a specific behavioral performance[19]. For example, the expression of full-length mRNA of the c-Fos gene is evident within 5–10 min after stimulation, with subsequently detectable c-Fos protein within 30–45 min[20].

The amygdala is a key structure in anxiety/fear regulation. This region is often studied in the context of emotion-processing and anxiogenic behavior, but the neuronal mechanisms that control these processes are still unclear[21]. The amygdala is rich in androgen receptors[22] while also containing both alpha and beta estrogen receptors[23]. The amygdala is affected by the circulating level of testosterone, and androgen receptors located in axons in this region play an important role in the rapid behavioral effects of androgens[24].

Although doubts regarding the existence of non-genomic steroid effects have disappeared, their role in anxiety and/or depressive behavior is not fully understood. In this study, we examined the behavioral changes induced by the steroid hormones testosterone and estradiol and explored their non-genomic effects on neurons in the amygdala, using c-Fos protein as an indicator. Specifically, the medial nucleus (MeA), the central nucleus (CeA), and the basolateral nucleus (BLA) of the amygdala were investigated.

MATERIAL AND METHODS

Animals

Thirty male Wistar rats (aged 15 weeks; AnLab Prague, Czech Republic) were housed in groups of six in polycarbonate cages (58 × 36 × 20 cm^3) under a 12-h light:12-h dark cycle (lights on at 07:00) at 25 ± 2°C and 55 ± 10% humidity. Food and water were available *ad libitum* in the cages. Rats were divided into four groups: control (CTRL; *n* = 8), castration (GDX; *n* = 8), castration + testosterone treatment (GDX + T; *n* = 7) and castration + estradiol (GDX + E; *n* = 7). The animal experiments were approved by the Ethics Committee of the Faculty of Medicine, Comenius University in Bratislava, Slovakia.

Surgery

At the age of 15 weeks, castration or sham operation was performed. Rats were anesthetized with ketamine and xylazine (100 mg/kg and 10 mg/kg) and both testes were extracted through a small incision at the posterior tip of the scrotum. Skin and subcutaneous tissue were sutured in two layers—subcutaneous tissue with absorbable Chirlac 5/0 (Chirmax GmbH, Ratingen, Germany) and skin with non-resorbable Chiralen suture 5/0 (Chirmax). Sham castration in the control group involved the extraction of both testes and epididymis followed by gentle replacement back into the scrotum with sutures as described above. Rats were allowed 2 weeks for recovery. No animals were excluded because of surgical complications.

Treatment

Animals were handled by the investigator every day before testing (weighing, food administration, removal from the cage, and transfer to another cage). Two weeks after surgery, sham-castrated animals were treated intramuscularly with olive oil (100 μL Oleum Olivae; VULM SK s.r.o., Slovakia), and castrated animals were treated with either olive oil, testosterone (5 mg/kg testosterone propionate T1875; Sigma Aldrich LLC, St. Louis, MO), or estradiol (0.5 mg/kg β-estradiol 17-propionate; 46556 Fluka, Sigma Aldrich). The doses of testosterone[9] and estradiol[25] were based on previous experiments. Hormonal supplementation and olive oil vehicle administration were performed by the same experimenter in the room where the animals were housed.

Behavioral Testing

All behavioral tests were conducted during the light phase of the cycle (09:00 – 12:00). Five minutes after application of olive oil, testosterone, or estradiol, rats completed a series of behavioral tests within 30 min in the following order: open-field test (5 min), novel object-recognition test (5 min), light-dark box test (5 min), and forced-swim test (3 min), without an inter-trial break. This time-window is generally accepted for non-genomic effects, since the genomic action of androgens and estrogens typically occurs after at least 30 min[26, 27]. The behavioral testing lasted for 5 consecutive days (6 animals per day).

Open-Field Test

The arena measured 100 × 100 cm^2 in area, 40 cm in height, and was placed in a dark room, with the middle of the maze well illuminated. The walls and floor of the arena were painted black inside and white outside. Rats were placed in the middle of the maze and allowed to freely explore for 5 min. The time spent in the center, near the walls, and in the corners of the maze was determined. Ethovision XT 8.5 tracking software (Noldus, Netherlands) was used to define a 40 × 40 cm^2 virtual central zone. More time spent in the illuminated center of the maze reflects less anxious behavior[28].

Simple Novelty Object Recognition

The arena was the same as that used in the open-field test. However, a new object was placed in one corner of the arena. Cone-shaped cups were used as target stimuli. The rats were allowed to move freely for 5 min and the time spent interacting with the new object was recorded. Sniffing or touching the object with the nose while running around the object was considered the criterion for interaction. More time interacting with the new object reflects less anxiety and higher exploration-derived behavior[29].

Light-Dark Box

The light-dark box arena was 80 × 40 cm^2 in area and 40 cm in height, divided into well-illuminated and dark halves, the latter covered by a roof. The two parts of the arena were separated by a black wall with an entrance. The rats were placed in the illuminated part facing the wall for 5 min and the time spent in the illuminated part and the number of entrances/exits to the dark half were recorded. More

time spent in the illuminated part of the box reflects less anxiety[30].

Forced-Swim Test

Depression-like behavior was assessed using the Porsolt forced-swim test. Rats were placed in a 80-cm tall Plexiglas cylinder filled with 24 ± 1°C water, from which the animals could not escape, for 3 min. Every 5 s, one of three forms of behavior was recorded: climbing, defined as rats trying to escape the cylinder; swimming, defined as rats performing circular movements; and immobility, defined as floating on the water without other movements. Immobility time corresponds to depression-like behavior[31].

Hormone Assays

After the last test, blood samples were obtained immediately after decapitation. The concentrations of testosterone and estradiol in plasma were determined using commercial ELISA kits (DRG Diagnostics, Marburg, Germany).

Immunohistochemistry

Immediately after behavioral testing, rats were anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine and rapidly decapitated. Brains were removed, frozen on dry ice, and stored at −80°C until sectioning. The right hemisphere from each rat was sectioned coronally on a Zeiss cryostat (Walldorf, Germany) at 30 μm; every third section was thaw-mounted on Superfrost Plus slides (Fisher, Chicago, IL) and fixed for 5 min in 4% formalin in phosphate-buffered saline (PBS, pH 7.4). Slides were then rinsed in PBS, heated in citrate buffer (pH 6.0) for 20 min in a microwave, and cooled for 20 min. After antigen retrieval, the slides were washed in PBS, blocked for 1 h with 1% BSA at room temperature, and incubated overnight with the primary antibody rabbit anti-c-Fos (ab7963, Abcam, Cambridge, UK, 1:1000) at 4°C. In negative controls, the primary antibody was omitted. The tissue was then rinsed in PBS and incubated with 0.3% hydrogen peroxide for 10 min, washed with PBS, and incubated with EnVision anti-rabbit for 30 min (K400, Dako , Carpinteria, CA). The slides were rinsed in PBS and the reaction visualized using DAB (K3468, Dako). After dehydration, the slides were coverslipped with Entellan (Merck, Darmstadt, Germany).

c-Fos-positive cells in the MeA, CeA, and BLA regions were visualized under an Olympus BX41 microscope with a 20× objective. Images were consistently taken from the same region according to the rat brain atlas[32]. Positive cells were counted in a 250 × 250 μm^2 field using ImageJ (NIH, Bethesda, MD) by an investigator blind to the treatment. Images were enhanced, subjected to thresholds, and measured. The enhancement improved contrast and removed background noise. Threshold treatment allowed distinction of immunoreactive cells from the background. Editing involved the removal of extraneous particles. An average count was used to calculate cell density. Only cells clearly distinguishable from the background were counted.

Statistical Analysis

Data are presented as mean ± standard error (SE), and were analyzed by one-way analysis of variance (ANOVA) with Tukey's *post-hoc* test. Significance was assigned to a value of $P < 0.05$.

RESULTS

We found higher testosterone levels in the GDX + T group than in the GDX group ($F = 76.74$, $P < 0.001$, Fig. 1A). Similarly, higher concentrations of estradiol were found in the GDX + E group than in the GDX group ($F = 66.86$, $P < 0.001$, Fig. 1B). There were also significant differences in testosterone levels between the GDX+T and GDX+E groups.

Behavioral Tests

In the open-field test, the GDX + E group spent more time in the central zone than the GDX group ($F = 3.55$, $P < 0.05$, Fig. 2A). Administration of testosterone had no effect on behavior. In simple novelty recognition, no significant differences were found between the groups ($F = 0.24$, $P = 0.86$, Fig. 2B). In the light-dark box test, both the GDX + T and GDX + E groups showed a trend of spending less time in the illuminated zone compared to the GDX group ($F = 2.87$, $P = 0.06$, Fig. 2C). In the forced-swim test, neither estradiol nor testosterone supplementation had any effect on the immobility time compared with the GDX group ($F = 1.11$, $P = 0.36$, Fig. 2D).

c-Fos-Expressing Neurons

c-Fos-stained neurons were visible in photomicrographs (Fig. 3). In the MeA, the number of c-Fos immunoreactive

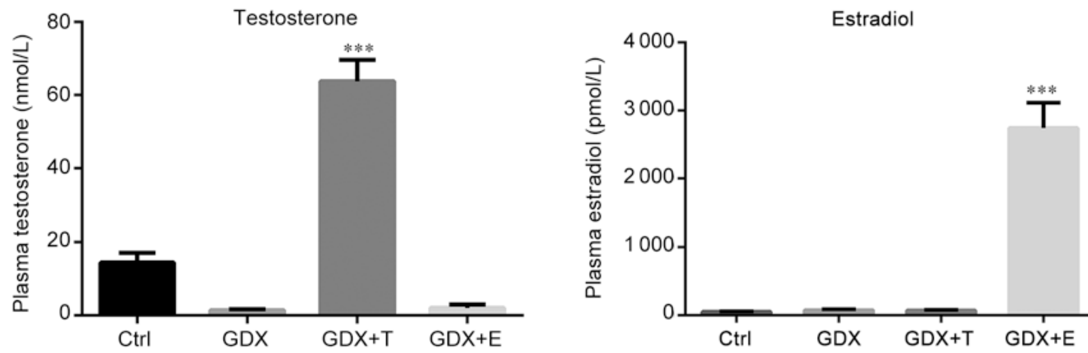

Fig. 1. Concentrations of testosterone and estradiol in plasma 30 min after treatment. Mean ± SE; ******P* <0.001 compared to GDX group. Ctrl, control group; GDX, castrated group; GDX + T, castrated group treated with testosterone; GDX + E, castrated group treated with estradiol.

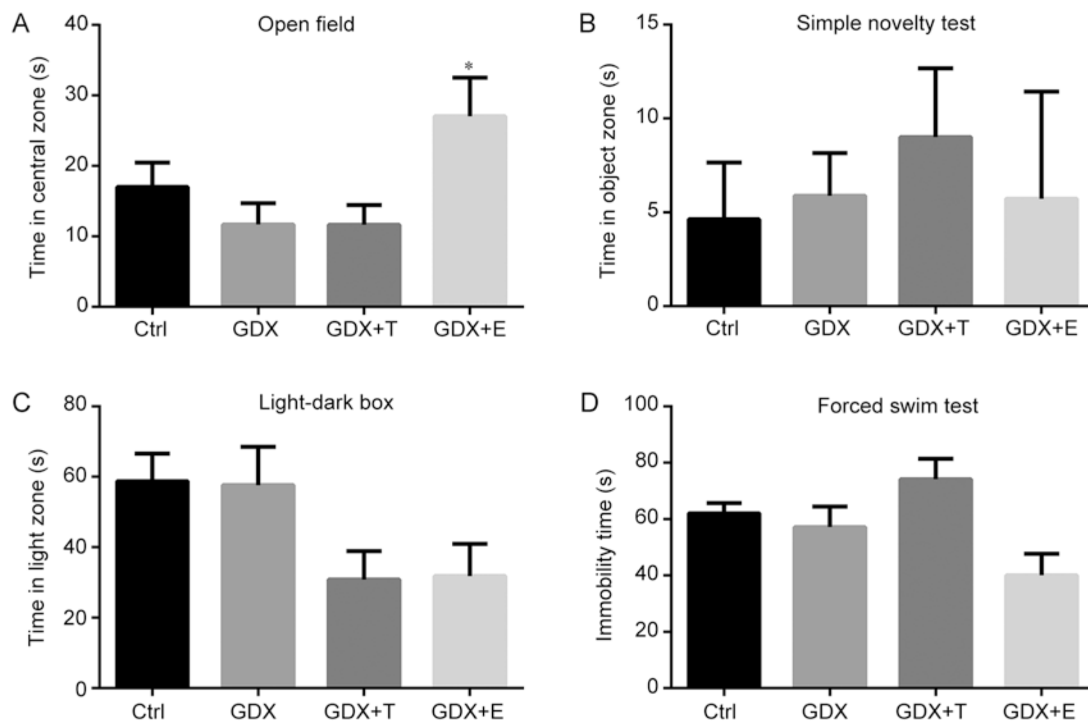

Fig. 2. Behavioral test results. A: Open-field test and time spent in the central zone. B: Simple novelty test and interaction with the new object. C: Light-dark box and time in the illuminated zone. D: Forced-swim test expressed as immobility time. Mean ± SE; ****P* <0.05 compared to GDX. Ctrl, control group; GDX, castrated group; GDX + T, castrated group treated with testosterone; GDX + E, castrated group treated with estradiol.

cells was higher in the GDX + T group than in the GDX group by 61% (F = 5.85, P <0.05, Fig. 4A). In the CeA and BLA, we found no differences in c-Fos cell numbers in the GDX + T and GDX + E groups compared to the GDX group (Fig. 4B and C).

DISCUSSION

Our results showed that estradiol had a rapid anxiolytic effect on male rat behavior in the open field test. In the other tests, we found no significant differences after estradiol supplementation. Application of testosterone

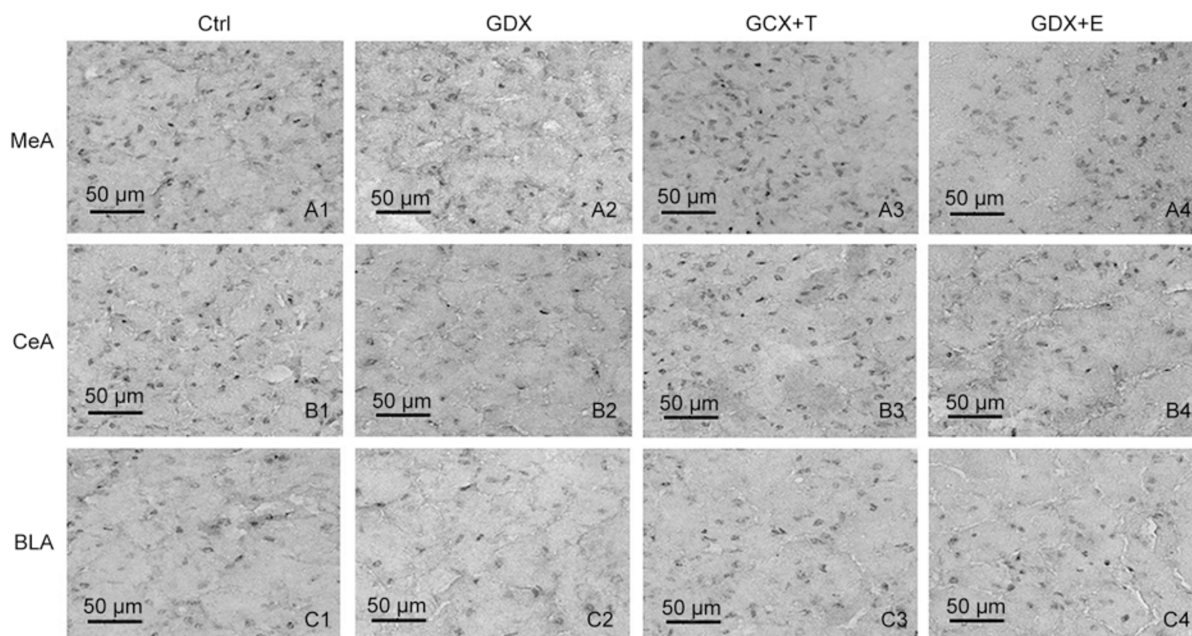

Fig. 3. Photomicrographs of neurons expressing c-Fos in the MeA (A1–A4), CeA (B1–B4), and BLA (C1–C4). MeA, medial nucleus; CeA, central nucleus; BLA, basolateral nucleus of the amygdala.

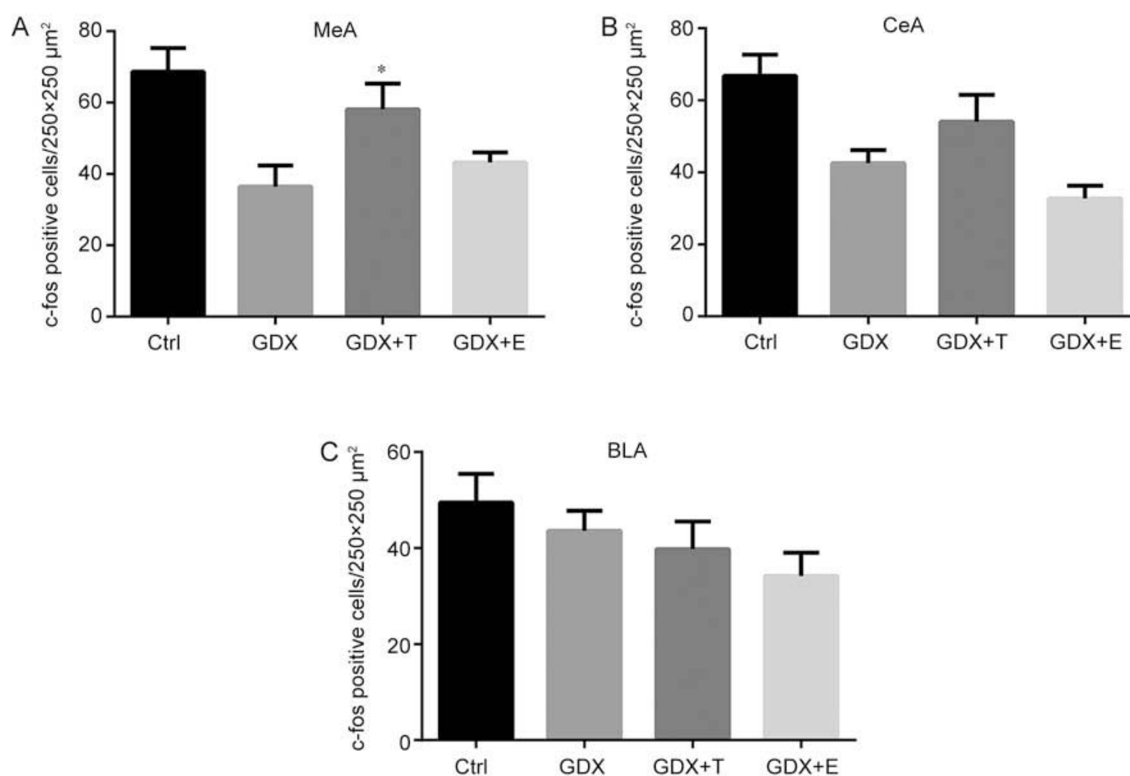

Fig. 4. Numbers of c-Fos-expressing neurons in the MeA (A), CeA (B), and BLA (C). MeA, medial nucleus; CeA, central nucleus; BLA, basolateral nucleus of the amygdala. Mean ± SE; **P* <0.05 compared to GDX group.

was ineffective in all behavioral tests. However, most of the behavioral studies dealing with testosterone have reported an anxiolytic effect[7, 9, 10]. A study by Aikey *et al.* showed that testosterone administration rapidly (30 min after application) reduces anxiety in male mice in the elevated plus maze. This anxiolytic effect is mediated by conversion of testosterone to reduced metabolites that interact with $GABA_A$ receptors. Also, dihydrotestosterone application results in anxiolytic effect, while estradiol does not[10]. Similar results were found in male rats. Gonzalez *et al.*[33] reported that castration of male rats is anxiogenic and testosterone treatment is anxiolytic. In contrast, Minkin *et al.*[34] found that treatment with nandrolone decanoate increases anxiety in gonadally intact and gonadectomized rats. Their study involved long-term (8-week) treatment, allowing for the study of genomic effects. In contrast, many authors have suggested that the genomic effects of testosterone on behavior are anxiolytic and mediated *via* the androgen receptor[7, 9, 10].

Estrogens can have direct non-genomic effects on neuronal membranes and thus affect neuronal activity. They can be produced locally by the aromatization of testosterone in presynaptic terminals and their production can be modulated within minutes by calcium-dependent phosphorylation[35]. There is evidence that estradiol induces anxiolytic behavior[16, 36]. The non-genomic effects of estradiol have been studied by Trainor *et al.*[37]. Estradiol has rapid effects on the behavior of male deer mice (*Peromyscus sp.*) in long (16 h light:8 h dark) and winter-like short days (8 h light:16 h dark). Estradiol injection into male *P. polionotus* experiencing short days increases aggressive behavior within 15 min[37], but there is no significant effect on behavior during long days. These results suggest that estradiol increases aggression during short days in mice by activation of a non-genomic mechanism, as it is generally thought that 15 min is insufficient for gene expression changes mediated by estrogen receptors[38]. Results from these studies indicate that estradiol can quickly influence behavior, which is in accord with our study. Furthermore, aggressive behavior could be associated with reduced anxiety.

c-Fos expression is a good indicator of neuronal activity. There is some evidence for a direct correlation between anxiety and c-Fos induction in brain areas, including the MeA, of young adult rats[39]. In our study, testosterone application to castrated rats increased c-Fos expression in the MeA without affecting the CeA and BLA. These results are not in line with our findings from behavioral testing, since administration of estradiol had no effect on the number of c-Fos-immunoreactive cells in the amygdala. However, non-genomic effects of steroids occurring within seconds to minutes after hormonal stimulation are too rapid for protein synthesis[40]. Finally, induction of c-Fos protein requires transcriptional activation. Thus, in some sense, the presence of c-Fos represents a genomic response to stimulation by sex steroids such as testosterone[41]. Kovacs and Sawchenko reported the highest c-Fos expression between 1 and 2 h after stimulation[42], which suggests that 30 min after steroid application is not sufficient time for quantification of the effect of steroids on the number of c-Fos-positive cells. Although c-Fos is often used as a marker for non-genomic action, the role of steroid receptors other than those in the intracellular milieu cannot be excluded in this rapid effect. There is evidence that non-genomic effects of steroids are mediated *via* estrogen and androgen receptors and this action can interact with slow genomic influence in the brain[3, 43]. This is indeed a major limitation of our study, and our further studies will use antagonists of androgen and estrogen receptors to distinguish between the genomic and non-genomic actions of steroids.

Furthermore, some work suggests that the rapid effect of testosterone is mediated *via* its aromatization into estradiol. Brain aromatase activity can quickly change, and this may rapidly influence the behavior of male mice[44, 45]. Some studies also suggest that the non-genomic effects of testosterone require high concentrations of this hormone[44]. Castration decreases testosterone concentrations in the brain to an undetectable level. Nevertheless, in this study, testosterone supplementation raised its concentration to ~3-fold the control level. On the other hand, injection of estradiol into castrated male rats increases the pre-copulatory and copulatory behavior of receptive females. This effect occurs 15 min after estradiol exposure. In contrast, application of testosterone is ineffective[46]. However, a short-latency effect of testosterone on copulatory behavior occurs when testosterone is applied to intact male rats[47]. The ineffectiveness of testosterone in the study of Cross and coauthors might have been due to the castrated status of the males. We found similar

effects on anxiety after testosterone administration. If the non-genomic effect of testosterone is mediated *via* aromatization, then 5 min (as shown in our study) would be an insufficient time-window for behavioral changes. On the other hand, applying estradiol to castrated males alleviated anxiety as indicated by increased time spent in the central area of the open field arena.

In conclusion, anxiolytic behavior was observed in estradiol-supplemented male rats, which suggest a rapid effect of estradiol. At least to some extent, this could be explained by the need for aromatization of testosterone to estradiol, which could require a longer time to take effect. Testosterone seemed not to have a direct effect on the anxiety level. Neither testosterone nor estradiol seemed to affect the depression-like behavior. Blockade of androgen and estrogen receptors in future studies should help to extend the time-window for non-genomic influences of sex steroids on anxiety behavior.

ACKNOWLEDGEMENTS

This work was supported by a VEGA grant (1/0406/13), the Ministry of Education of Slovak Republic, the APVV-0753-10 and the UK/384/2013.

Received date: 2014-06-24; Accepted date: 2014-10-01

REFERENCES

[1] Maggi A, Ciana P, Belcredito S, Vegeto E. Estrogens in the nervous system: mechanisms and nonreproductive functions. Annu Rev Physiol 2004, 66: 291–313.

[2] McEwen BS. Non-genomic and genomic effects of steroids on neural activity. Trends Pharmacol Sci 1991, 12: 141–147.

[3] Foradori CD, Weiser MJ, Handa RJ. Non-genomic actions of androgens. Front Neuroendocrinol 2008, 29: 169–181.

[4] Heinlein M. Plasmodesmata: dynamic regulation and role in macromolecular cell-to-cell signaling. Curr Opin Plant Biol 2002, 5: 543–552.

[5] Carrier N, Kabbaj M. Extracellular signal-regulated kinase 2 signaling in the hippocampal dentate gyrus mediates the antidepressant effects of testosterone. Biol Psychiatry 2012, 71: 642–651.

[6] Buddenberg TE, Komorowski M, Ruocco LA, Silva MA, Topic B. Attenuating effects of testosterone on depressive-like behavior in the forced swim test in healthy male rats. Brain Res Bull 2009, 79: 182–186.

[7] Hodosy J, Zelmanova D, Majzunova M, Filova B, Malinova M, Ostatnikova D, *et al.* The anxiolytic effect of testosterone in the rat is mediated via the androgen receptor. Pharmacol Biochem Behav 2012, 102: 191–195.

[8] Frye CA, Walf AA. Depression-like behavior of aged male and female mice is ameliorated with administration of testosterone or its metabolites. Physiol Behav 2009, 97: 266–269.

[9] Bitran D, Kellogg CK, Hilvers RJ. Treatment with an anabolic-androgenic steroid affects anxiety-related behavior and alters the sensitivity of cortical GABAA receptors in the rat. Horm Behav 1993, 27: 568–583.

[10] Aikey JL, Nyby JG, Anmuth DM, James PJ. Testosterone rapidly reduces anxiety in male house mice (*Mus musculus*). Horm Behav 2002, 42: 448–460.

[11] Edinger KL, Frye CA. Testosterone's analgesic, anxiolytic, and cognitive-enhancing effects may be due in part to actions of its 5alpha-reduced metabolites in the hippocampus. Behav Neurosci 2004, 118: 1352–1364.

[12] Fernandez-Guasti A, Martinez-Mota L. Anxiolytic-like actions of testosterone in the burying behavior test: role of androgen and GABA-benzodiazepine receptors. Psychoneuroendocrinology 2005, 30: 762–770.

[13] Frye CA, Rhodes ME, Dudek B. Estradiol to aged female or male mice improves learning in inhibitory avoidance and water maze tasks. Brain Res 2005, 1036: 101–108.

[14] Walf AA, Frye CA. A review and update of mechanisms of estrogen in the hippocampus and amygdala for anxiety and depression behavior. Neuropsychopharmacology 2006, 31: 1097–1111.

[15] Galea LA, Lee TT, Kostaras X, Sidhu JA, Barr AM. High levels of estradiol impair spatial performance in the Morris water maze and increase 'depressive-like' behaviors in the female meadow vole. Physiol Behav 2002, 77: 217–225.

[16] Morgan MA, Pfaff DW. Effects of estrogen on activity and fear-related behaviors in mice. Horm Behav 2001, 40: 472–482.

[17] Walf AA, Frye CA. Rapid and estrogen receptor beta mediated actions in the hippocampus mediate some functional effects of estrogen. Steroids 2008, 73: 997–1007.

[18] Filova B, Ostatnikova D, Celec P, Hodosy J. The effect of testosterone on the formation of brain structures. Cells Tissues Organs 2013, 197: 169–177.

[19] Kovacs KJ. Measurement of immediate-early gene activation-c-fos and beyond. J Neuroendocrinol 2008, 20: 665–672.

[20] Hoffman GE, Smith MS, Verbalis JG. c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems. Front Neuroendocrinol 1993, 14: 173–213.

[21] Tye KM, Prakash R, Kim SY, Fenno LE, Grosenick L, Zarabi H, *et al.* Amygdala circuitry mediating reversible and

bidirectional control of anxiety. Nature 2011, 471: 358–362.
[22] Rubinow DR, Schmidt PJ. Androgens, brain, and behavior. Am J Psychiatry 1996, 153: 974–984.
[23] Mitra SW, Hoskin E, Yudkovitz J, Pear L, Wilkinson HA, Hayashi S*, et al.* Immunolocalization of estrogen receptor beta in the mouse brain: comparison with estrogen receptor alpha. Endocrinology 2003, 144: 2055–2067.
[24] Sarkey S, Azcoitia I, Garcia-Segura LM, Garcia-Ovejero D, DonCarlos LL. Classical androgen receptors in non-classical sites in the brain. Horm Behav 2008, 53: 753–764.
[25] Walf AA, Frye CA. Estradiol reduces anxiety- and depression-like behavior of aged female mice. Physiol Behav 2010, 99: 169–174.
[26] Michels G, Hoppe UC. Rapid actions of androgens. Front Neuroendocrinol 2008, 29: 182–198.
[27] Nabekura J, Oomura Y, Minami T, Mizuno Y, Fukuda A. Mechanism of the rapid effect of 17 beta-estradiol on medial amygdala neurons. Science 1986, 233: 226–228.
[28] Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 2003, 463: 3–33.
[29] Ennaceur A, Michalikova S, Chazot PL. Models of anxiety: responses of rats to novelty in an open space and an enclosed space. Behav Brain Res 2006, 171: 26–49.
[30] Crawley J, Goodwin FK. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol Biochem Behav 1980, 13: 167–170.
[31] Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 1977, 229: 327–336.
[32] Paxinos G., Watson C. The Rat Brain in Stereotaxic Coordinates, 6th ed. New York: Academic Press, 2007: 456.
[33] Gonzalez MI, Farabollini F, Albonetti E, Wilson CA. Interactions between 5-hydroxytryptamine (5-HT) and testosterone in the control of sexual and nonsexual behaviour in male and female rats. Pharmacol Biochem Behav 1994, 47: 591–601.
[34] Minkin DM, Meyer ME, van Haaren F. Behavioral effects of long-term administration of an anabolic steroid in intact and castrated male Wistar rats. Pharmacol Biochem Behav 1993, 44: 959–963.
[35] Balthazart J, Baillien M, Ball GF. Rapid control of brain aromatase activity by glutamatergic inputs. Endocrinology 2006, 147: 359–366.
[36] Diaz-Veliz G, Alarcon T, Espinoza C, Dussaubat N, Mora S. Ketanserin and anxiety levels: influence of gender, estrous cycle, ovariectomy and ovarian hormones in female rats. Pharmacol Biochem Behav 1997, 58: 637–642.
[37] Trainor BC, Finy MS, Nelson RJ. Rapid effects of estradiol on male aggression depend on photoperiod in reproductively non-responsive mice. Horm Behav 2008, 53: 192–199.
[38] Vasudevan N, Pfaff DW. Membrane-initiated actions of estrogens in neuroendocrinology: emerging principles. Endocr Rev 2007, 28: 1–19.
[39] Duncan GE, Knapp DJ, Breese GR. Neuroanatomical characterization of Fos induction in rat behavioral models of anxiety. Brain Res 1996, 713: 79–91.
[40] Heinlein CA, Chang C. The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. Mol Endocrinol 2002, 16: 2181–2187.
[41] Nagypal A, Wood RI. Region-specific mechanisms for testosterone-induced Fos in hamster brain. Brain Res 2007, 1141: 197–204.
[42] Kovacs KJ, Sawchenko PE. Sequence of stress-induced alterations in indices of synaptic and transcriptional activation in parvocellular neurosecretory neurons. J Neurosci 1996, 16: 262–273.
[43] Vasudevan N, Pfaff DW. Non–genomic actions of estrogens and their interaction with genomic actions in the brain. Front Neuroendocrinol 2008, 29: 238–257.
[44] Nyby JG. Reflexive testosterone release: a model system for studying the nongenomic effects of testosterone upon male behavior. Front Neuroendocrinol 2008, 29: 199–210.
[45] Cornil CA, Taziaux M, Baillien M, Ball GF, Balthazart J. Rapid effects of aromatase inhibition on male reproductive behaviors in Japanese quail. Horm Behav 2006, 49: 45–67.
[46] Cross E, Roselli CE. 17beta-estradiol rapidly facilitates chemoinvestigation and mounting in castrated male rats. Am J Physiol 1999, 276: R1346–1350.
[47] Malmnas CO. Short-latency effect of testosterone on copulatory behaviour and ejaculation in sexually experienced intact male rats. J Reprod Fertil 1977, 51: 351–354.