Original Article

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Effects of Exercise and Estrogen on Anxiety-like Behaviors in Ovariectomized Mice

R. Alimohammadi (MSc)[1], S. Naderi (MSc)[1], M. Allahtavakoli (PhD)*[1]

1.Physiological and Pharmacological Research Center, Rafsanjan University of Medical Sciences, Rafsanjan I.R.Iran

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ABSTRACT

BACKGROUND AND OBJECTIVE: Anxiety is a major symptom of menopause caused by loss of ovarian activity. Anxiety increases the intensity of vasomotor symptoms in menopausal women. This study aimed to compare the effects of exercise and estrogen on anxiety level of ovariectomized mice.

METHODS: This empirical study was conducted on 28 mice (weight: 25-35 grams) divided into four groups of seven, including ovariectomy, ovariectomy and exercise, ovariectomy and estrogen (40 mg/kg of estradiol valerate), and ovariectomy combined with exercise and estrogen. Animals were initially ovariectomized and one week later, they were placed on treadmills to run at medium intensity for 30 minutes per day. Intervention continued for five days per week, and after four weeks, anxiety was evaluated using elevated plus-maze.

FINDINGS: In this study, estrogen significantly increased the percentage of open arm entry (OAE) compared to ovariectomy group (22.13±4.72 vs. 4.91±3.18, respectively) ($p<0.05$). In addition, combination of estrogen and exercise significantly increased open arm time (OAT) compared to ovariectomy group (46.19±6.82 vs. 4.91±3.18, respectively) ($p<0.001$). However, no significant difference was observed between exercise and estrogen groups. Also, exercise alone increased OAE compared to ovariectomy group (24.54±3.18 vs. 13.79± 3.23, respectively) ($p<0.05$). Percentage of OAE in groups of estrogen, exercise and combined exercise and estrogen was 30.61±1.25, 24.54±3.18 and 46.08±1.04, respectively, which was indicative of no significant difference. However, estrogen and combined estrogen and exercise significantly increased OAE compared to ovariectomy group ($p<0.001$).

CONCLUSION: According to the results of this study, similar to estrogen, exercise could reduce the anxiety induced by ovariectomy in mice.

KEY WORDS: Estrogen, Exercise, Anxiety, Menopause.

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***Corresponding Author: M. Allahtavakoli (PhD)**

Address: Physiological and Pharmacological Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, I.R.Iran.

Tel: +98 391 5234003

E-mail: allahtavakoli@gmail.com

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Introduction

Ageing causes ovaries to gradually lose their ability of responding to gonadotropins, which results in decreased function of ovaries. This process discontinues the menstrual cycle leading to menopause in women. Menopause lowers the level of sex hormones and increases the concentration of follicle-stimulating hormone and luteinizing hormone (1, 2).

Reduced level of estrogen due to menopause leads to several complications in women, including hot flashes, night sweats, fatigue, irritability and tremors, memory loss, reduced executive brain function, dizziness, anxiety and depression. Menopausal women may experience at least a few of these problems, also known as vasomotor symptoms (3).

Anxiety is a common disorder among menopausal women (3). It is a natural state and an adaptive component of response to acute stress, which is triggered when personal integrity of an individual is at risk. However, if unbalanced in terms of severity or chronicity or accompanied with any specific risks to the health of individual, anxiety is considered as a non-adaptive response and even mental disorder (4). On the other hand, anxiety could increase the severity of vasomotor symptoms associated with menopause (5). Several factors are involved in the occurrence of anxiety, such as lifestyle, genetics, gender and hormonal levels, especially sex hormones (3, 4).

According to the literature, anxiety is more prevalent among women compared to men. Recent studies have reported the prevalence of anxiety to be 30.5% among women and 19.2% among men (6). High concentration of estrogen hormone is known to reduce the level of anxiety (6, 7).Several reports have indicated that menopausal women may frequently experience anxiety due to decreased estrogen levels (8). Anxiety caused by menopause could be treated by hormone replacement therapy (9, 10). However, long-term use of estrogen is likely to increase the risk of endometriosis, breast cancer and cardiovascular diseases in menopausal women (11, 12).

Researchers have been investigating alternative treatments for estrogen therapy due to the side effects of synthetic estrogen consumption. Regular exercise is associated with numerous advantages, such as memory reinforcement and prevention of different disorders, such as anxiety (13). Previous studies have confirmed that exercise could eliminate negative emotions and boost positive senses. Furthermore, regular exercise recovers mental disorders such as stress and anxiety (14). Reports have suggested that exercise enhances neurogenesis in gyrus dentatus of hippocampus in rats, which plays a pivotal role in the regulation of anxiety processes (15).

According to the study by Patki et al., moderate exercise on treadmill could reduce anxiety-like behaviours in rats through influencing the hippocampus (16). In another study, Greenwood et al. indicated that exercise could increase resistance against environmental stressors (17). Regular exercise protects body organs against the debilitating effects of internal stress mechanisms. Correspondingly, Binder et al. reported that there was an increase in the time spent in the open arms of an elevated plus maze in mice with four weeks of exercise training.

As a result, severity of anxiety-like behaviors reduced in these animals (18). Exercise could remarkably reduce anxiety and unlike synthetic estrogen, it is associated with no side effects. This study aimed to compare the effects of exercise and estrogen therapy on the reduction of anxiety in ovariectomized mice.

Methods

Experimented animals: This empirical study was conducted on 28 female mice weighing 25-35 grams. Animals were divided into four groups of seven and kept in separate cages under tranquil conditions within a photocycle of 12 hours of light and 12 hours of darkness. Subjects were maintained at temperature of 1±21°C and had free access to sufficient food and water. Study protocol was approved by the Ethics Committee of Rafsanjan University of Medical Sciences, Iran. For experiments, animals were divided into the following groups:

1)Ovariectomy: Mice in this group were ovariectomized and administered with one ml/kg of saline via gavage for one month.

2)Ovariectomy and estrogen: Animals in this group were ovariectomized and administered with 40 µg/kg of estradiol valerate via gavage for one month.

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3)Ovariectomy and exercise: Mice in this group were initially ovariectomized. Afterwards, they were placed on treadmills to run at intensity of 18 meter/minute five days per week for one month.

4)Ovariectomy, exercise and estrogen: Animals in this group were initially ovariectomized. Afterwards, they were placed on treadmills to run at intensity of 18 meter/minute five days per week, and 40 µg/kg of estradiol valerate was administered via gavage for one month (19, 20).

Drugs used in experiments: In this study, we used estradiol valerate manufactured by Aboureihan Pharmaceutical Company, Iran.

Method of ovariectomy (menopause induction): For ovariectomy, mice were weighed and administered with 90 mg/kg of ketamine and 4.5 mg/kg of xylazine via intraperitoneal injection. After anaesthesia, abdomen of animals was shaved, and surgery area was sterelized. To find ovaries, both sides of abdominal section (between the second and third breast, next to thigh muscle) were cut open. following that, fallopian tube was burnt using a cautery device, and ovaries (red follicular tissue attached to oviduct tube) were slowly seperated and removed.

In the next stage, internal and external layers were sutured seperately, and 22000 µ.i/kg of penicillin was injected into the thigh muscle of animals. Finally, animals were returned to their cages to recover (20, 21).

Assessment of ovariectomy accuracy: After three days, vaginal smears were obtained from animals for six consecutive days. To do so, a few drops of normal saline were discharged on the vagina of mice using a bulb and removed afterwards.

Drops were placed on glass slides and examined using a microscope. If there were no ferny patterns in the smear, ovariectomy was performed with success (21).

Physical exercise: For physical exercise, we used electrical treadmills manufactured by ITTC Life Science Co. Animals were placed on treadmills to run at intensity of 6-9 meter/minute after getting accustomed to the exercise (22). Exercise was carried out for half an hour at medium intensity of 18 meter/minute daily.

This intervention was performed on animals in the exercise group five days per week for four consecutive weeks. Mice without exercise were placed on turned-off treadmills. Body weight of animals was measured every three days in order to assess possible anxiety caused by exercise (19).

Examination of anxiety-like behaviors: To evaluate anxiety-like behaviors in subjects, we used elevated plus-maze (EPM) (Figure 1) based on the model proposed by Pellow et al. This device is made of wood and has four arms in the form of a plus sign. Dimensions of open and closed arms are 50×10 with a 40-cm wall at both sides and end of closed arms. To prevent animals from falling off the maze, a glass edge (one cm) has been installed on both sides and end of open arms.

All four arms are directed towards a central area with dimensions of 10×10 cm. EPM was located at 50 cm distance from the ground, and animals were placed in the central area of the maze to face an open arm. Proper lighting was adjusted using a 100 W lamp located at 120 cm away from the center of the maze. While animals were freely moving in the maze (five minutes), we videotaped the number of times animals entered each open and closed arm. Moreover, time spent in closed and open arms of EPM was recorded and measured (23). Entry into each arm was defined when all four legs of animals were placed in open or closed arms, and total time spent in each arm was determined in the same manner. Percentage of open arm entry (OAE) and open arm time spent (OAT) in EPM for each animal was calculated using the following formula:

$$OAE\% = \left(\frac{OAE}{OAE + CAE}\right) \times 100$$

$$OAT\% = \left(\frac{OAT}{OAT + CAT}\right) \times 100$$

Significant increase in either of the aforementioned parameters was indicative of reduced anxiety although OAE % has lower sensitivity in recording anxiety-like or anti-anxiety behaviors of animals compared to OAT % (24). Data analysis was performed using one-way analysis of variance (ANOVA) and Tukey's post-hoc test, and P value of less than 0.05 was considered significant.

Results

Effect of exercise on anxiety-like behaviors: According to our findings, exercise significantly

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increased OAE compared to ovariectomy-only group (2313.79±3.18 vs. 24.54±3.18, respectively) (*p<0.05) (fig 1), which denotes the anti-anxiety effect of exercise. However, no significant difference was observed between mice in the exercise and estrogen group in this regard.

Effect of estrogen on anxiety-like behaviors: Administration of estradiol valerate (40 µg/kg) resulted in a significant increase in OAT compared to ovariectomy group (22.13±4.72 vs. 4.791±3.18, respectively) (p<0.05) (fig 2). Moreover, estrogen could significantly increase OAE compared to ovariectomy group (30.61±1.25 vs. 13.79±3.23, respectively) (p<0.01), which emphasizes the anti-anxiety effect of this drug. However, no significant difference was observed in the exercise group in this regard.

Effect of combined exercise and estrogen on anxiety-like behaviors: Administration of estradiol valerate (40 µg/kg) with exercise resulted in a significant difference in OAT compared to ovariectomy group (46.19±6.82 vs. 4.91±3.18, respectively) (p<0.001). In addition, combination of estradiol valerate (40 µg/kg) and exercise significantly increased OAE compared to ovariectomy group (46.8±1.04 vs. 13.79±3.23, respectively) (p<0.001), which denotes the anti-anxiety effect of this combination. However, no significant difference was observed between mice receiving combined exercise and estrogen and those receiving estrogen only and exercise only.

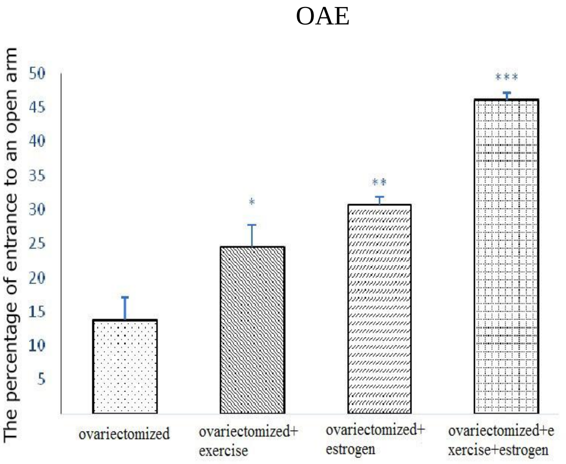

Figure 1. Effects of Exercise, Estrogen and Combination of Exercise and Estrogen on OAE

(Data presented as Mean±SEM (N=7); *p<0.05, **p<0.01,***p<0.001 Compared to Ovariectomy Group)

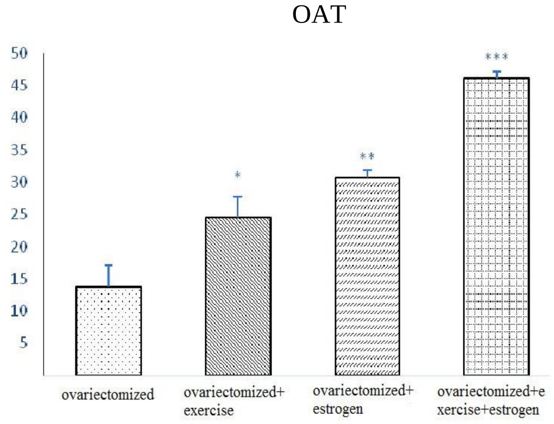

Figure 2. Effects of Exercise, Estrogen and Combination of Exercise and Estrogen on OAT

(Data presented as Mean±SEM (N=7);*p<0.05, ***p<0.001 Compared to Ovariectomy Group)

Discussion

According to the results of this study, treadmill exercise at intensity of 18 meter/minute for half an hour per day (five days a week for four weeks) could reduce OAE factor of anxiety-like behaviors caused by ovariectomy in mice. It is noteworthy that estrogen exerted similar effects on these behaviors. On the other hand, combination of estrogen and exercise had a more significant effect on the reduction of anxiety-like behaviors in both OAE and OAT factors compared to estrogen and exercise alone.Reduced anxiety followed by regular exercise could be associated with changes in the hippocampus.

As observed in adult rodents, running could increase the number of new stimulating neurons in gyrus dentatus, while stimulating the production of dendritic branches on these neurons, throughout the hippocampal circuit. In rodents, ventral hippocampus is directly involved in processing behaviors such as stress and anxiety (25).

According to the literature, exercise enhances neurogenesis in gyrus dentatus of hippocampus in mice leading to an increase in growth factors, such as insulin-like growth factor-1 and brain-derived neurotrophic factor, which play a pivotal role in the regulation of anxiety-like processes (26). Recent findings suggest that mice who receive treadmill exercise for five consecutive weeks tend to have reduced levels of adrenocorticotropin (ACTH) and corticosterone, which results in the reduction of anxiety-like behaviors in these animals (27). Exercise enhances the function of hypothalamic-

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pituitary axis to control subsequent stressors. This process is associated with changes in the release of corticosterone, termination of response to this hormone, or both these parameters together. Changes in the release of corticosterone from the adrenal gland could be caused by altered sensitivity of this gland to ACTH, as well as changes in the release of ACTH from the pituitary gland (28). Previous research has indicated that estrogen could also be effective in reducing anxiety through affecting the amygdala and hippocampus (29, 30). Furthermore, estrogen could decrease anxiety and stress through E2 receptors stimulation of hypothalamic-pituitary-adrenal axis (31). In one study, FULK et al. performed 45 minutes of treadmill exercise on rats at medium intensity (five times per week for ten weeks), and the intervention led to a significant reduction in anxiety-like behaviors of animals (32).

These findings are consistent with the results obtained by the present study. In another research, Uysal et al. reported that treadmill exercise could decrease plasma levels of corticosterone in mice causing a significant reduction in anxiety (33). Exercise is associated with physiological and behavioral effects, including improved learning ability, reduced anxiety-like behaviors, neurogenesis and angiogenesis, increased neurotrophic factors and changes in various signaling molecules. In their study, Salam et al. concluded that running on a wheel in the Rotarod device (two weeks) resulted in the significant reduction of anxiety-like behaviors in male mice (34). Furthermore, Vollert et al. claimed that rats receiving regular exercise had normal corticosterone concentration and reduced anxiety-like behaviors compared to those without exercise (35). Similarly, findings of Haydari et al. suggested that exercise could decrease the level of anxiety in mice (36). Results obtained in the current study indicated that exercise alone could reduce anxiety-like behaviors in ovariectomized mice, which is consistent with the results of the aforementioned studies.

Therefore, considering the harmful effects of synthetic estrogen, exercise could be a beneficial alternative to reduce anxiety in menopausal women. In conclusion, since physical exercise comes in a variety of forms and effects on the brain, it is recommended that further research be conducted as to discover the most efficient types of exercise and their influence on molecular mechanisms involved in the reduction of anxiety. Comparison of these findings with estrogen therapy could yield beneficial results for clinical situations.

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References

1.Barrett KE ,Ganong WF. Ganong's review of medical physiology. 23rd ed. NewYork: McGraw-Hill Medical. 2010.

2.Honari N, Pourabolli I, Hakimizadeh E, Roohbakhsh A, Shamsizadeh A, Vazirinejad R, et al. Effect of vitex agnus castus extraction on anxiety-like behaviors in ovariectomized rats. J Babol Univ Med Sci. 2012;14(5):29-35.[In Persian]

3.Hardy R, Kuh D. Change in psychological and vasomotor symptom reporting during the menopause. Soc Sci Med. 2002;55(11):1975-88.

4.Millan MJ. The neurobiology and control of anxious states. Prog Neurobiol. 2003;70(2):83-244.

5.Freeman EW, Sammel MD, Lin H, Gracia CR, Kapoor S, Ferdousi T. The role of anxiety and hormonal changes in menopausal hot flashes. Menopause. 2005;12(3):258-66.

6.Lim L, Ng TP, Chua HC, Chiam PC, Won V, Lee T, et al. Generalised anxiety disorder in Singapore: prevalence, co-morbidity and risk factors in a multi-ethnic population. Soc Psychiatry Psychiatr Epidemiol. 2005;40(12):972-9.

7.Seeman MV. Psychopathology in women and men: focus on female hormones .Am J Psychiatry. 1997;154(12):1641-7.

8.Arushanian E, Chernysheva E. [A comparative evaluation of the effect of removal of the epiphysis and damage to the amygdala on the behavioral reactions of rats]. Zh Vyssh Nerv Deiat Im I P Pavlova. 1995;46(4):762-8.

9.Arpels JC. The female brain hypoestrogenic continuum from the premenstrual syndrome to menopause. A hypothesis and review of supporting data. J Reprod Med. 1996;41(9):633-9.

10.Sherwin BB. Estrogen and cognitive functioning in women: lessons we have learned. Behav Neurosci. 2012;126(1):123-7.

11.Barnabei VM, Cochrane BB, Aragaki AK, Nygaard I, Williams RS, McGovern PG, et al. Menopausal symptoms and treatment-related effects of estrogen and progestin in the Women's Health Initiative. Obstet Gynecol. 2005;105(5 Pt 1):1063-73.

12.Hammond C. Women's concerns with hormone replacement therapy--compliance issues. Fertil Steril. 1994;62(6 Suppl 2):157S-60S.

13.da Silva R, de Morais A, de Melo J, Macedo P, Costa L, Hornsby MBO. Neonatal exercise prevents anxiety-related behavior and improves episodic memory in adult but not in aged rats. FASEB J. 2015;29(Suppl 1):840.14.

14.McArthur JA, Effects of repeated voluntary or forced exercise on rat brain serotonergic systems. [Undergraduate Theses]. USA: University of Colorado. paper.843.

15.Droste SK, Gesing A, Ulbricht S, Muller MB, Linthorst AC, Reul JM. Effects of long-term voluntary exercise on the mouse hypothalamic-pituitary-adrenocortical axis. Endocrinology. 2003;144(7):3012-23.

16..۱۶Patki G, Solanki N, Atrooz F, Ansari A, Allam F, Jannise B, et al. Novel mechanistic insights into treadmill exercise based rescue of social defeat-induced anxiety-like behavior and memory impairment in rats. Physiol Behav. 2014;130:135-44.

17.Greenwood BN, Loughridge AB, Sadaoui N, Christianson JP, Fleshner M. The protective effects of voluntary exercise against the behavioral consequences of uncontrollable stress persist despite an increase in anxiety following forced cessation of exercise. Behav Brain Res. 2012;233(2):314-21.

18.Binder E, Droste SK, Ohl F, Reul JM. Regular voluntary exercise reduces anxiety-related behaviour and impulsiveness in mice. Behav Brain Res. 2004;155(2):197-206.

19.Ding YH, Young CN, Luan X, Li J, Rafols JA, Clark JC, et al. Exercise preconditioning ameliorates inflammatory injury in ischemic rats during reperfusion. Acta Neuropathol. 2005;109(3):237-46.

20.Clouthier S, Wicha M. Ketamine/Xylazine containing anesthesia for mouse surgery preparation. Univ Michigan Health Syst. 2012;1-2.

21.Emerton K, Hu B, Woo A, Sinofsky A, Hernandez C, Majeska R, et al. Osteocyte apoptosis and control of bone resorption following ovariectomy in mice. Bone. 2010;46(3):577-83.

[DOI: 10.22088/jbums.17.12.40]

[DOR: 20.1001.1.15614107.1394.17.12.6.5]

22. Kim SE, Ko IG, Kim BK, Shin MS, Cho S, Kim CJ, et al. Treadmill exercise prevents aging-induced failure of memory through an increase in neurogenesis and suppression of apoptosis in rat hippocampus. Exp Gerontol. 2010;45(5):357-65.
23. Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods. 1985;14(3):149-67.
24. Zarrindast MR, Farahvash H. Effects of GABA-ergic drugs on penile erection induced by apomorphine in rats. Psychopharmacology(Berl). 1994;115(1-2):249-53.
25. Schoenfeld TJ, Rada P, Pieruzzini PR, Hsueh B, Gould E. Physical exercise prevents stress-induced activation of granule neurons andenhances local inhibitory mechanisms in the dentate gyrus. J Neurosci. 2013;33(18):7770-7.
26. Wang DC, Chen TJ, Lin ML, Jhong YC, Chen SC. Exercise prevents the increased anxiety-like behavior in lactational di-(2-ethylhexyl) phthalate-exposed female rats in late adolescence by improving the regulation of hypothalamus-pituitary-adrenal axis. Horm Behav. 2014;66(4):674-84.
27. Hare BD, Beierle JA, Toufexis DJ, Hammack SE, Falls WA. Exercise-associated changes in the corticosterone response to acute restraint stress: evidence for increased adrenal sensitivity and reduced corticosterone response duration. Neuropsychopharmacology. 2014;39(5):1262-9.
28. Frye CA, Walf AA. Estrogen and/or progesterone administered systemically or to the amygdala can have anxiety-, fear-, and pain-reducing effects in ovariectomized rats. Behav Neurosci. 2004;118(2):306-13.
29. Lund TD, Rovis T, Chung WC, Handa RJ. Novel actions of estrogen receptor-β on anxiety-related behaviors. Endocrinology. 2005;146(2):797-807.
30. 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(6):1097-111.
31. Fulk L, Stock H, Lynn A, Marshall J, Wilson M, Hand G. Chronic physical exercise reduces anxiety-like behavior in rats. Int J Sports Med. 2004;25(01):78-82.
32. Uysal N, Kiray M, Sisman A, Camsari U, Gencoglu C, Baykara B, et al. Effects of voluntary and involuntary exercise on cognitive functions, and VEGF and BDNF levels in adolescent rats. Biotech Histochem. 2015;90(1):55-68.
33. Salam JN, Fox JH, Detroy EM, Guignon MH, Wohl DF, Falls WA. Voluntary exercise in C57 mice is anxiolytic across several measures of anxiety. Behav Brain Res. 2009;197(1):31-40.
34. Vollert C, Zagaar M, Hovatta I, Taneja M, Vu A, Dao A, et al. Exercise prevents sleep deprivation-associated anxiety-like behavior in rats: potential role of oxidative stress mechanisms. Behav Brain Res. 2011;224(2):233-40.
35. Haydari S, Miladi-Gorji H, Mokhtari A, Safari M. Effects of voluntary exercise on anxiety-like behavior and voluntary morphine consumption in rat pups borne from morphine-dependent mothers during pregnancy. Neurosci Lett. 2014;578:50-4.

[DOR: 20.1001.1.15614107.1394.17.12.6.5]
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