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Role of noise and music as anxiety modulators: Relationship with ovarian hormones in the rat

Begoña Escribano[a,*], Ismael Quero[b], Montserrat Feijóo[a], Inmaculada Tasset[a], Pedro Montilla[a,1], Isaac Túnez[a]

[a] Department of Cell Biology, Physiology and Immunology, Faculty of Veterinary Medicine, Córdoba University, Campus de Rabanales s/n, 14071 Cordoba, Spain
[b] Department of Biochemistry and Molecular Biology, Faculty of Medicine, Maimónides Institute of Biomedical Research of Córdoba (IMIBIC), Córdoba University, Cordoba, Spain

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ABSTRACT

This work aims to verify the role of noise and music as anxiety modulators and their relationship with ovarian hormones. For this purpose, female Wistar rats were used and treated, forming different experimental groups, with 17-β-estradiol, progesterone, allopregnanolone, finasteride (inhibitor of the 5-α-reductase) and faslodex (inhibitor of estrogen receptors), with the rats being previously ovariectomized in the case of the first three hormones. All the groups were submitted to stimuli of silence, white noise (sound that has all frequencies) and music (sonata for 2 pianos K. 448 by W.A. Mozart), and to two valuation tests of the anxiety levels: elevated plus-maze and light-dark box transition. In this study, the anxiogenic effect of noise and the anxiolytic effect of music were verified. Both exerted their effects separately from the ovarian steroid hormones, although the influence of progesterone and allopregnanolone increased the anxiolytic power of the music. 17-β-estradiol was the least influential of the ovarian hormones and its effects might be concealed by the intervention of the other two (allopregnanolone and progesterone).

1. Introduction

Environmental enrichment is a dynamic process in which changes to structures and husbandry practices are made with the goal of increasing behavioural choices to animals and drawing out their species appropriate behaviour and abilities, thus enhancing animal welfare (BHAG, 1999, provided by Valerie Hare). Bloomsmith et al. (1991) identified five major types of enrichment (social, occupational, physical, sensory and nutritional), each of which can be subdivided. Actually, within sensory enrichment, auditory stimulation by means of music has been investigated in different species, including humans.

Contradictory results in domestic animals (Dávila et al., 2011) have been shown for auditory stimulation. Uetake et al. (1997) found positive effects from auditory enrichment on behaviour in dairy cows, whereas Cloutier et al. (2000) observed negative effects on stress in pigs, and Christensen and Knight (1975) found no significant effect on growing performance in meat-type chickens. A recent article suggested that music may not execute an effective enrichment in captive gibbons (Wallace et al., 2013), although auditory enrichment by means of classical music was a reliable method for reducing stress levels in several species (Chikahisa et al., 2007; Guetin et al., 2009; Dávila et al., 2011; Marzban et al., 2011; Davis and Jones, 2012; Johnson et al., 2012; Lin et al., 2013).

Abbreviations: OVX, ovariectomized rats; SO, sham operated rats; 17βe, 17-β-estradiol; P, progesterone; allo-p, allopregnanolone; s.c., subcutaneous.

* Corresponding author. Tel.: +34 957 212026/+34 957 218685; fax: +34 957 212186/34 957 212002.
E-mail address: am1esdub@uco.es (B. Escribano).

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The power of music in anxiety tests has been argued in the rat. Lemercier (2000) concluded that environmental enrichment, such as by short-duration music, decreases the rat's reactivity in housing spaces but only has a little influence when rats are taken out to be handled and has no influence on the results obtained during an anxiety test (Elevated Plus-Maze). However, Rauscher (2006) show a neurophysiological basis for a Mozart effect in rats to complete a T-maze more quickly if they had been reared listening to a Mozart piano sonata, a conclusion that is contested by Steele (2006), who argued that the rats were deaf to most of the notes of the sonata. However, exposure to Mozart's music early in life can increase BDNF (brain-derived neurotrophic factor) concentration in the hippocampus in rats (Marzban et al., 2011).

Chikahisa et al. (2007) hypothesized that exposure to Mozart's music will reduce anxiety levels in mice and that gender differences might be observed related to ovarian steroid hormones. The ovarian steroid hormones, estrogen and progesterone, have been well documented as having an association with anxiety levels (Joffe and Cohen, 1998; Schmidt et al., 2000; Koss et al., 2004; Saavedra et al., 2006). The results of Chikahisa et al. (2007) suggest that exposure to music reduces anxiety levels in mice, and ovarian steroids, mainly progesterone, may be involved in this effect.

The anxiolytic-like action of estradiol and progesterone administration in ovariectomized (OVX) female rats has been demonstrated in an elevated plus-maze test (Bitran et al., 1993; Koss et al., 2004), but not in a light/dark transition test. To be specific, the psychotropic effects observed after progesterone administration are due to the bioconversion of progesterone to allopregnanolone, which subsequently augments GABA(A) receptor-mediated function (Bitran et al., 1993), via a nongenomic mechanism (Bitran et al., 1995). This bioconversion is mediated by 5-α-reductase enzyme, which modulates the estrous cycle variations, producing a lower anxiety in proestrous for the elevated plus maze test than in diestrous, when the enzyme dose is lower (Koonce et al., 2012). The possible anxiolytic action of direct allopregnalonone administration in OVX rats has been assayed in the burying behaviour test (Fernandez-Guasti and Picazo, 1995), but not in the elevated plus maze and light-dark box transition tests.

We therefore hypothesized in rats that: (i) the Sonata for 2 pianos K. 448 by W.A. Mozart could reduce anxiety levels in two behavioural tests (elevated plus maze and light–dark box transition tests), in opposition to white noise and silence. Also, we hypothesized (ii) a role in anxiety reduction of the estrogens, progesterone and allopregnalonone administration in OVX rats in the elevated plus maze and light–dark box transition tests. Finally, we assayed as anxiolytics (iii) the role of ovarian steroid hormones with two inhibitors, one of 5-α-reductase and another of estrogen receptors, in rats with intact ovaries.

2. Material and methods

2.1. Animals and treatments

Female Wistar rats (200–250 g) three months old and with regular ovarian cycles were used in this study. The rats, housed in groups of three animals in a plastic cage (524 × 274 × 150 mm), were fed ad lib (Purina®, Barcelona) and maintained in a 12-h light/dark cycle (lights on at 08:00 h) at a controlled temperature (21–23 °C). All behavioural testing was completed between 13:00 and 17:00 h, and rats were used only once for each type of behavioural test. The experiments were approved by the Bioethics Committee of Córdoba University and carried out according to the guidelines of the Directive of 24 November 1986 (86/609/ECC) approved by the European Communities Council.

The experiments carried out were:

Experiment 1*:* In this study the role of Mozart's music, noise and silence, in anxiety was investigated. Control rats were randomly assigned to one of three groups: (i) silence group, (ii) white noise group and (iii) Mozart music group, with $n = 6$ rats per group. In the music or white-noise group, rats were exposed to music or white noise from 30 to 45 min before the behavioural tests and also during the test (Chikahisa et al., 2007). Music (Mozart's 2 piano sonata, K. 448) was played repeatedly on a CD player, and white noise (sound that has all frequencies) was reproduced by a stereo radio, which was not tuned to any particular station (frequency of 300–10,000 Hz) both apparatus situated at 70–100 cm from the rats (Lin et al., 2013). The silence condition was maintained in the same room as the music and white noise groups, where there was no sound except ambient noise. The sound level of the silence group was 55 dB, and 65 dB for music and white noise in the home cages and in the behavioural apparatus with the sound level measured by a decibelmeter (PCE-222®, PCE Ibérica S.L., Spain).

Experiment 2*:* This was designed to verify the role of ovarian steroid hormones in the anxiogenic effects of white noise and the anxiolytic ones of music detected in experiment 1. Rats were ovariectomized (OVX, $n = 18$) or sham operated (SO, $n = 18$). After a 14 day recovery period, the rats were randomly assigned to one of six groups ($n = 6$ per group): (i) SO-silence; (ii) SO-white noise; (iii) SO-music (Mozart's 2 piano sonata, K. 448); (iv) OVX-silence; (v) OVX-white noise; (vi) OVX-music. The three stimuli (silence, white noise and music) were applied with the same protocol of experiment 1.

Experiment 3*:* To establish which ovarian steroid hormones were involved in anxiety levels, in experiment 3 in OVX rats, they were injected with 17-β-estradiol (17βe), progesterone (P) and allopregnanolone (allo-p). 0.50 mg/kg weight/day (w.d) for 17βe, 0.50 mg/kg w.d for P and 250 μg/kg w.d for allo-p (metabolite of the progesterone) were injected subcutaneously (s.c.) in OVX rats during the three days before the behavioural testing. Dimethylsulphoxide (DMSO) was administered s.c. to control animals. Behavioural testing was performed 1 h after last administration s.c. to animals. 12 groups were established ($n = 6$ per group): (i) OVX + DMSO-silence; (ii) OVX + DMSO-white noise; (iii) OVX + DMSO-music; (iv) OVX + 17βE-silence; (v) OVX + 17βE-white noise; (vi) OVX + 17βE-music (Mozart's 2 piano sonata, K. 448); (vii) OVX + P-silence; (viii)) OVX + P-white noise; (ix) OVX + P-music; (x) OVX + allo-p-silence; (xi) OVX + allo-p-white noise; (xii) OVX + allo-p-music.