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# Interaction between estradiol and 5-HT$_{1A}$ receptors in the median raphe nucleus on acquisition of aversive information and association to the context in ovariectomized rats

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ABSTRACT

The median raphe nucleus (MRN) is related to stress resistance and defensive responses, a crucial source of serotonergic neurons that project to prosencephalic structures related to stress and anxiety. Estrogen receptors were identified in this mesencephalic structure. It is possible that the estrogen action is related to serotonin effect on somatodendritic 5-HT$_{1A}$ receptors, inhibiting the function of serotonergic neurons and thus preventing of the stress effect and inducing anxiolysis. So, in order to evaluate these aspects, female *Wistar* rats were ovariectomized and 21 days later were given a direct microinjection of estradiol benzoate (EB) (1200 ng) into the MRN, preceded by microinjections of saline or WAY100.635 (100 ng), a 5-HT$_{1A}$ receptor antagonist. Immediately after the two microinjections, the ovariectomized rats were conditioned with an aversive event (foot shock) session in a Skinner box. Twenty-four hours later, they were exposed to the same context in a test session for 5 min for behavioral assessment: freezing, rearing, locomotion, grooming, and autonomic responses (fecal boluses and micturition). EB microinjection in the MRN prior to the exposure of animals to the foot shocks in the conditioning session did not alter their behavior in this session, but neutralized the association of the aversive experience to the context: there was a decrease in the expression of freezing and an increased rearing activity in the test session. This effect was reversed by prior microinjection of WAY100.635. In conclusion, EB acted on serotonergic neurons in the MRN of the ovariectomized rats, impairing the association of the aversive experience to the context, by co-modulating the functionality of somatodendritic 5-HT$_{1A}$.


## 1. Introduction

Compelling evidence indicates that females are more susceptible to anxiety than males (Leach et al., 2008; McLean and Anderson, 2009; NIMH, 2012), mainly in phases of the hormonal cycle when serum levels of estrogen are low, such as during premenstrual and peri-menopausal periods, or after bilateral oophorectomy (Altemus, 2006; Bekker and van Mens-Verhulst, 2007; McHenry et al., 2014; Rocca et al., 2008; Seeman, 1997). During these periods, there appears to be an increase in sensitivity to stressors (Andreano and Cahill, 2010; Kask et al., 2008; Ossewaarde et al., 2010). Experimental and clinical studies (in mice, rats, and humans) have demonstrated that estrogen replacement treatment minimizes the effect of stressors, decreases anxiety (Walf and Frye, 2010; Walf et al., 2009; Wharton et al., 2013), and affects the functionality of the serotonergic system (Amin et al., 2005; Borrow and Cameron, 2014; Genazzani et al., 2007; Lasiuk and Hegadoren, 2007; McEwen, 2002).

The median raphe nucleus (MRN) is considered to be a crucial source of serotonergic neurons that project to prosencephalic structures related to stress and anxiety, such as the dorsal hippocampus (Azmitia and Segal, 1978; McKenna and Vertes, 2001). These anatomic analyses support theoretical assumptions that the MRN-dorsal hippocampus pathway is a critical component in stress resistance (Deakin and Graeff, 1991) and in anxiogenesis (Andrade et al., 2013) by integrating Gray's "behavioral inhibition system" (Gray and McNaughton, 2000).

Several studies have demonstrated the effects of stimulation or

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inactivation of the MRN on anxiety (for review see Andrade et al., 2013). More specifically, serotonergic neurons from the MRN were involved in the manifestation of freezing in contextual conditioning (Andrade et al., 2005; Avanzi and Brandão, 2001; Avanzi et al., 1998; Fendt and Fanselow, 1999; Luyten et al., 2011; Silva et al., 2002, 2004), an anxiety test which involves aversive conditioning and spatial context (Fanselow, 2000; Orsini et al., 2013). In addition, there is a wealth of experimental evidence indicating that the dorsal hippocampus, the main projection of the MRN, facilitates learning contextual characteristics in which conditioning occurred (Fanselow, 2000; Gewirtz et al., 2000; Gupta et al., 2001; Maren et al., 1997; Maren and Holt, 2000).

Estrogen receptors have been identified in neurons located in the MRN (Leranth et al., 1999; Alves et al., 2000), indicating a role for estrogen in this location. It is possible this role is to potentiate the action of serotonin on somatodendritic 5-$HT_{1A}$ receptors, inhibiting the function of serotonergic neurons (McEwen, 2002; Inoue et al., 2014), and causing anxiolysis (Andrade et al., 2005, 2009). One study showed that the microinjection of estradiol benzoate (EB: β-estradiol 3 benzoate) into the MRN of ovariectomized rats decreased the manifestation of freezing in the same context as that in which the animals received foot shocks (Andrade et al., 2009). This effect was reversed by prior injection of WAY100.635, a 5-$HT_{1A}$ receptor antagonist. In this case, the pharmacological manipulations were conducted 24 h after the aversive conditioning session, immediately before exposure to the context (test session). Thus, EB blocked the association between the aversive experience and the context.

The present study aimed to evaluate whether the estradiol microinjected into the MRN before exposure to an aversive stimulus (foot shocks) would impair the acquisition of aversive information and the association with context, and if the 5-$HT_{1A}$ receptors would be involved in this effect. The hypothesis of this investigation was that estradiol could contribute for the decrease of the aversive conditioning process, minimizing anxiety-like behavior in ovariectomized rats by modulating the function of the serotonergic neurons in MRN.

## 2. Materials and methods

### 2.1. Animals

Normal cycling female *Wistar* rats, weighing 200 g and that were at least 2 months old, were housed, five animals in one polypropylene cage (41 × 34 × 17 cm) with wood shavings on the floor, for 7 days until ovariectomy. After stereotaxic surgery, the animals were housed in pairs. The rats were maintained on a 12 h light-dark cycle (7:00–19:00, 50 lux) in a temperature-controlled room (21 ± 2 °C) and given free access to food and water throughout the experiment, except during testing. The animals were handled three times a week to clean the cages.

Procedures were approved by the research ethics committee of São Paulo State University (Process 553/2009; CEP 015/2009) and were conducted in conformity with the Brazilian Society of Neuroscience and Behavior Guidelines for Care and Use of Laboratory Animals, which are in compliance with international laws and policies. All efforts were made to minimize animal suffering.

### 2.2. Drugs

Estradiol benzoate (β-estradiol 3-benzoate; Sigma, USA) was dissolved in sesame oil (Sigma, USA) (1200 ng). WAY100.635 (N-[2-(4-[2-Methoxyphenyl]-1-piperazinyl)ethyl]-N-2-pyridinylcyclohexanecarboxamide) Sigma, USA, a selective 5-$HT_{1A}$ receptor antagonist, was dissolved in saline (100 ng). Control animals received the same volume of the respective vehicle (sesame oil or saline).

### 2.3. Surgeries

All females were bilaterally ovariectomized under tribromoethanol anesthesia (250 mg/kg, IP; Aldrich, USA). Fourteen days later, a 15 mm guide cannula was implanted into the brain of the animals at a 20° angle, with its tip remaining 1.5 mm above the injection site. This procedure was performed on rats fastened to a stereotaxic instrument (David-Kopf, USA) under the same anesthetic described above, with the addition of a local anesthetic of 2% xylocaine. The following coordinates from the atlas of Paxinos and Watson (2007) were used: anteroposterior = −7.8 mm; lateral = +2.9 mm; depth = −7.5 mm, taking the bregma as reference. The cannula was fixed to the skull with acrylic resin and a stainless steel screw.

At the end of the surgery, all animals were injected (IM) with 0.2 ml of antibiotic preparation (benzylpenicillin and streptomycin;

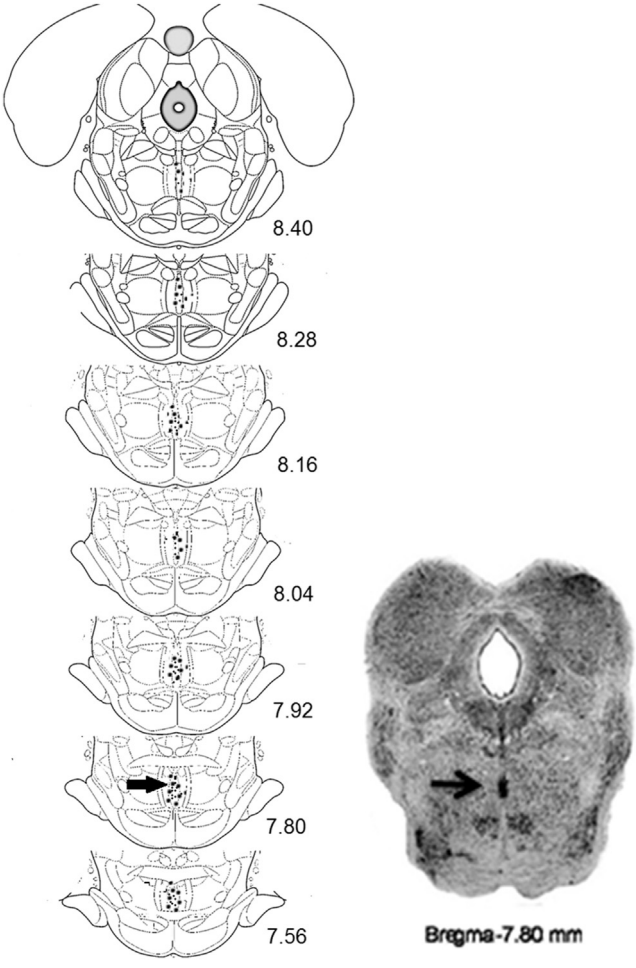


**Fig. 1.** Diagrammatic representation of coronal sections through the rat brain (mm posterior to bregma) showing the location of injection sites into MRN (Paxinos and Watson, 2007). The number of points in the figures is less than the total number of rats included in the analysis (n = 41) because of several overlaps (on the left side). Photomicrograph of typical injection site (indicated by arrow) in the MRN (on the right side).